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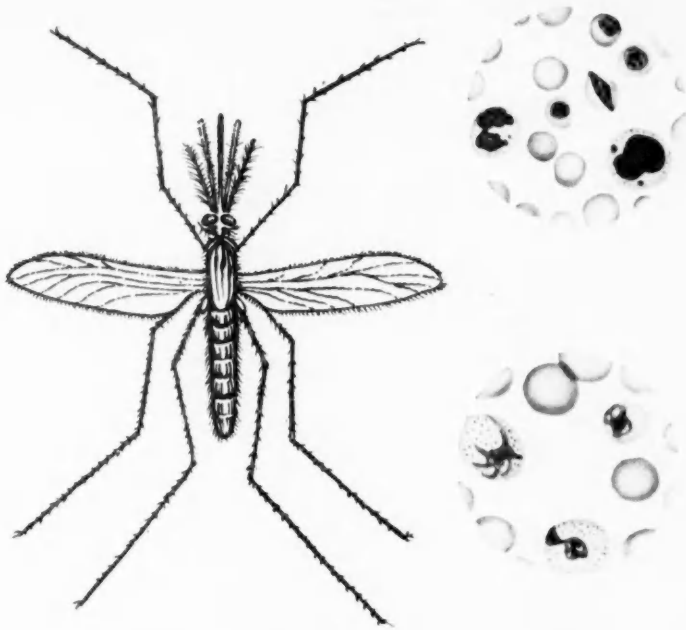

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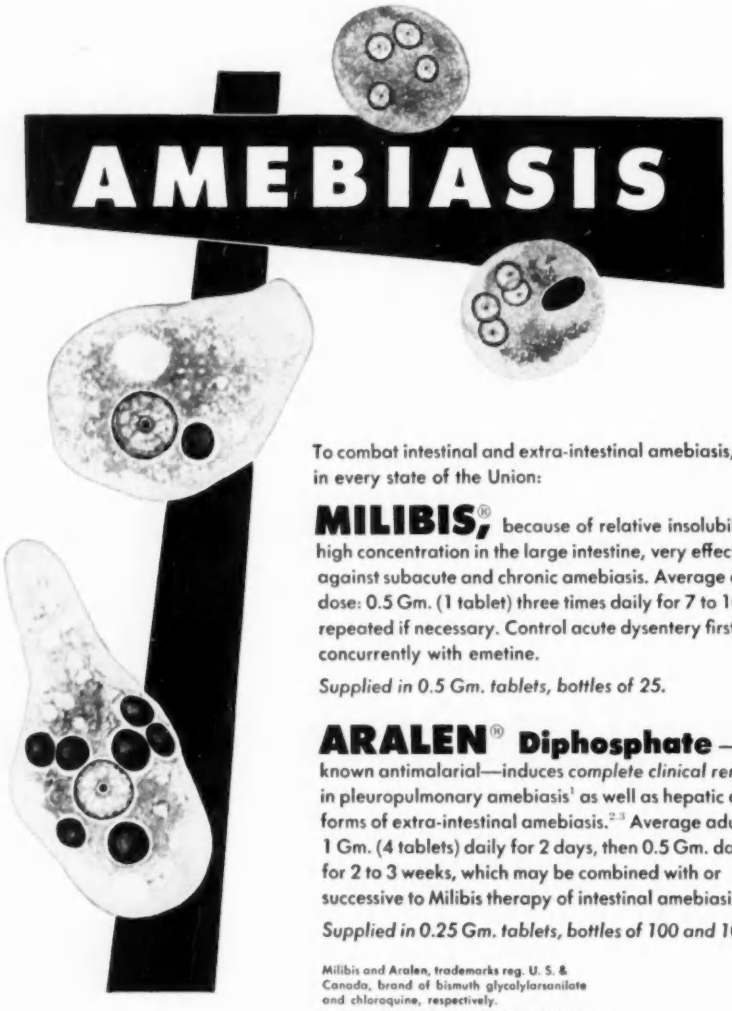

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2. Conan, N. J., Jr.: *Am. Jour. Med.*, 6:309, Mar., 1949.
3. Emmett, J.: *J.A.M.A.*, 141:22, Sept. 3, 1949.

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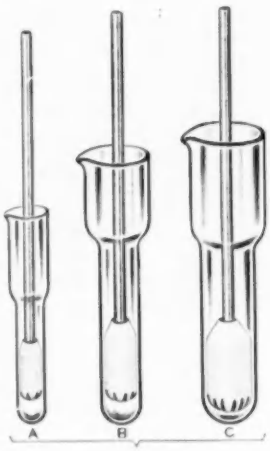

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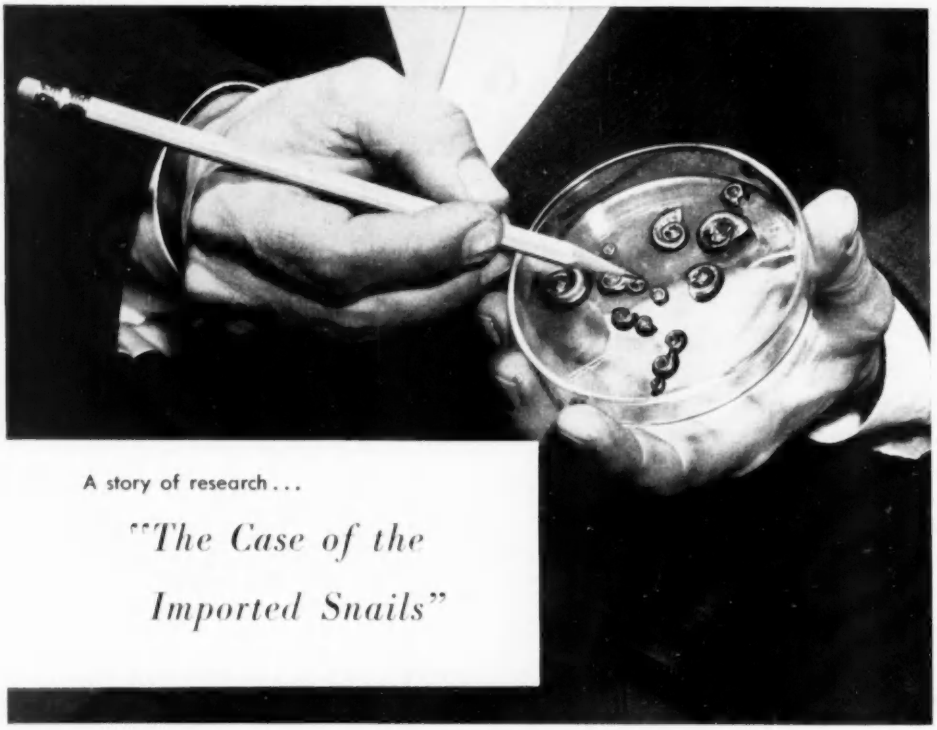

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1. Anderson, H. H., et al., Fumagillin in Amebiasis, Amer. J. Trop. Med. & Hyg., 1:552, July, 1952.

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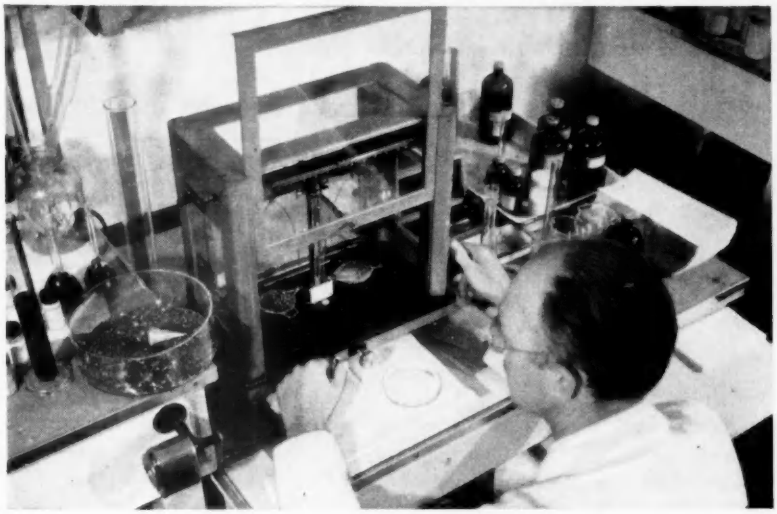

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1. Rednar, G. A.: South. M. J. 46:298 (March) 1953.
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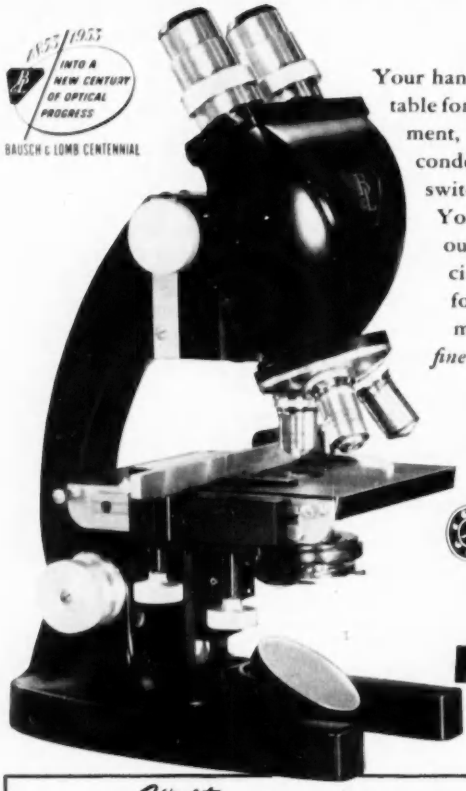

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FINANCING SANITARY WORKS IN THE TROPICS—A CHALLENGE[1]

ABEL WOLMAN

The Seventeenth Annual Charles Franklin Craig Lecture, before the American Society of Tropical Medicine and Hygiene, November 15, 1952

Not quite twenty years ago, your speaker had the pleasure of watching a master at work on the identification of a tropical disease which had already attacked over 500 persons in a North American city. A profound student of amebic dysentery, he moved from a position of doubt as to the true nature of the epidemic disease to one of rapid conviction as his tools of diagnosis and powers of clinical observation came into play. This demonstration of scientific acumen in Dr. Craig I have never forgotten.

Nor have I forgotten the modesty and proficiency with which he participated in the investigating commission's deliberations. From that field study, a major axiom appeared: that epidemic disease must have multiple causes, other than the specific organism. In the Chicago epidemic, aside from the presence of the amebic dysentery cysts, the striking collateral phenomenon in causation was the cloudy climate of administrative control of water-sewage relationships in two hotels.

The theme of this paper stems in part from that early association. It is difficult to express adequately an appreciation of the distinction and honor which your Society bestows upon me in lending to this contribution the distinguished name of so scholarly an investigator in tropical medicine as Dr. Craig.

Many observers have pointed out that tropical diseases differ in no important sense from the diseases which occur in many other parts of the world. Historically, the diseases of the tropics have merely outlived their opportunities in the rest of the world. Their decline in temperate zones has been the result of artificial measures of protection, much of which has been in environmental sanitation. The lessons learned from these successes in the temperate zones may be applied with some adjustments to the diseases which still plague the inhabitants of the tropics.

The elimination of typhoid fever and other enteric diseases, the building out of insect-borne diseases and the general modification of the physical environment for the protection of man are reasonably well understood disciplines. The professional worker in the tropics, however, must take these accepted principles and determine how they may be most successfully applied to the issues which confront him in a large part of the world.

When it is found that these accomplishments are in arrears as compared with the results so far attained in more favored areas, one searches naturally for some

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key to the failures. This key certainly does not rest in the absence either of scientific information or of diagnosis of local condition. By and large, principles for disease control and methods for environmental sanitation correction are reasonably well understood. What are the obstacles which bar the way to introduction of these methods and to wider participation in these promised successes?

John E. Gordon (1952) has recently pointed out that disease is the result of an ecologic process. It stems from a mutual relationship of various living organisms in an environment and their reaction to animate and inanimate surroundings. He includes in this ecological world all of the factors affecting the wellbeing of the species, not only "the agent of the disease but the intricate complex of the environment." This concept of disease causation gives us a cue as to the problem in the tropics. The multiple factors of food, of physical environment, of rates of reproduction, of economic and social status, all make for a dynamic equilibrium which at the moment may be unfavorable to maximum survival and health.

THE PROBLEM OF MONEY

Of these factors, population growth and economic status perhaps are two of the most significant restraints in the advancement of environmental sanitation.

Marston Bates (1952) has recently epitomized part of this conclusion by the comment: "Disease in the tropics is fostered more by poverty than by climate." Standard of living in the tropics, so often parallel with underdeveloped countries in general, is usually at low ebb. The introduction of sanitary facilities into such a population at once poses significant fiscal difficulties. This discussion will be devoted primarily to the major disabilities in underdeveloped countries due to the lack of financial resources. Of the population problem little will be said, since my ignorance of solutions to this issue is even greater than that in the financial sphere.

MacDonald (1950) in England, an experienced observer of the tropical scene, has emphasized the same conclusion when he stated several years ago that "We can control all the plagues in the tropics and exterminate many of them. The speed and degree of success with which we do this does not depend upon fresh advances in technical knowledge, but on the administrative arrangements to put our knowledge into practice."

Even in that vast area of mosquito borne disease control, Russell and his associates (1946) express an equally optimistic view that "adequate technique for control is available and that it is financially possible is beyond dispute, whatever myopic revenue officials may put down on minute papers."

We need not debate, therefore, that scientific knowledge is sufficiently far in advance of application to make it possible for us to reduce or even to eliminate many of the environmental diseases, provided we could produce the economic and social climate essential for the introduction of our well understood techniques. Money, therefore, becomes almost the major key to disease eradication in many of the areas of which we speak. It is not the sole source of hope, because the development of informed professional workers, of improved legislative sanctions

and of wider and more efficient administrative machinery are all essential parts of major effort. In sanitary works, however, the unavailability of money represents one of the great deterrents to progress.

THE RESOURCES

The greater part of this discussion will be devoted to this particular feature of public health effort, a function to which the health officer and the engineer too frequently devote little or no serious attention.

The questions which must be posed in this connection are simple ones: is money available in the tropical countries; can it be channeled into sanitary works; are the resources so inadequate that the only hope for development in these areas rests upon external gifts, grants-in-aid or other forms of largesse?

Consideration here will be devoted primarily to a review of the availability of resources within the country itself. The financing of sanitary works on the basis of gifts of any kind is ruled out, first because such a technique is increasingly unattractive to the recipient governments and secondly, as a long range form of financing, it is precarious to say the least. Some way must be found by which the governments of tropical countries will rely increasingly upon their own strengths and will progressively develop fiscal techniques by which indigenous resources will be channeled more and more into the important sanitary works of the country. The rate at which this use of money will move can only be determined by the growing financial strength of the country.

What is the present situation with respect to money in the underdeveloped countries? Fortunately a great deal of study has been devoted to this problem by a number of agencies. The International Bank for Reconstruction and Development has accumulated much experience in this area of interest. The Institute of Inter-American Affairs has shown high ingenuity and imagination in exploring and in developing local fiscal resources in Latin American countries. The United Nations (1950, 1951) through a long series of studies by its Department of Economic Affairs has disclosed a number of interesting features of financing in underdeveloped countries. The individual countries themselves have, of course, subjected their problems to intensive scrutiny. The Government of India, in the last five years has made intensive studies of its problems in this connection, both through its Planning Commission (1951) and through its Ministry of Health. The latter has issued a comprehensive report of its Environmental Hygiene Committee (1950) which devotes no inconsiderable part of its deliberations to the same problem.

Throughout all of these official and semiofficial reviews the recurrent challenge of finance is perhaps the most striking single issue to appear. In the Environmental Hygiene Report (1950) in India, the following indicative comment appears, "Water supply is the most crying need of villagers. It can be provided by capital expenditures. . . . If some financial wizard can suggest a way of raising this money, we will harness all the resources of public health engineering to achieve this to perfection." This search for the financial wizard is universal in

the underdeveloped countries. Is there any reasonable hope that this search might be successful in the not too distant future? Some optimism is possible; what does that optimism rest upon?

John H. Adler (1952) of the Economic Department of the International Bank has recently reviewed this whole problem with reference to government, since in most of these countries government is the important major source of local capital formation. It is his judgment that the two factors which limit severely the level of public and private investment are first and predominantly the level of private savings and the other the lack of an organized capital market through which savings can be channeled from the private saver to the financing of public investment.

Holdings of public debt by individuals are extremely small through Asia and the Far East. In virtually all of these countries the study indicates the major problem is how to devise incentives for greater savings and investments. In many countries the hoarding of savings is particularly significant and high expecially in two areas, South and Southeast Asia and in the Middle East. In these latter it is estimated the private gold hoards are sometimes as large as 10 per cent of the total national income.

Hoarding by governments as well is not an unusual characteristic because of the requirements they have set up that savings bank deposits must be backed by 100 per cent of foreign exchange.

These practices by private citizen and by government are considered to represent sources of investment money which for the moment cannot be pried loose, either because of lack of confidence in the stability of governments or by the unattractiveness of low interest rates from public investment.

MAKING MONEY AVAILABLE

All of these studies suggest ways of inducing individuals to enter the public market by one or more of the following procedures for increased use of savings:

1. Increased interest rates by institutions. For the most part, these amounts are restricted to only 2 or 3 per cent, insufficient to attract investment.
2. By the creation of better savings institutions.
3. By the development of well managed and regulated stock markets.
4. By attracting higher income classes to the diversion of their savings from less to more useful public purposes.
5. By reducing the private investment in land and real estate in metropolitan urban areas.
6. By a general reduction of mal-investment through improved education and governmental guidance.
7. By the compulsory reduction of conspicuous consumption by middle and higher income classes. In general, it is thought that this might be accomplished by taxation on luxury items.
8. By using the idle resources of the community, in the form of unemployed labor and some materials on spare time work on capital improvements. This type

of effort has been successfully employed in some of the Latin American countries, particularly in the construction of sanitary works.

One cannot review these studies in various parts of the world without realizing that in many countries sanitary works are possible of accomplishment through the existing resources of the countries, provided both government and people could be persuaded that their money would be intelligently and honestly planned and spent. One of the striking deficiencies in many of the countries is the absence of adequate facilities for safe deposit of voluntary savings. Almost every investigator, however, stresses the point that a fundamental consideration in making these funds available is the safety of the deposit. It is not surprising, but it is worth emphasizing, that confidence in government institutions is so often at low ebb that one of the writers is prompted to record the economic axion that "confidence is a tender growth."

The application of ingenuity to finance is also stressed and the suggestion is even made that in some of these countries the use of a lottery form of savings for public works should be seriously considered. The first Washington monument in the United States was built in Baltimore by such use of a lottery, but the method has long given way to more sophisticated fiscal procedures.

Another significant feature of all the inventories is the conclusion that taxation has not yet reached a burdensome level in many of the countries under discussion. The possibilities of general and special taxes are still relatively unexplored. The device used to a great extent in the United States of special assessments and of special tax authorities for such works is just beginning to be applied.

The high rate of interest is, of course, a symptom, not only of the lack of confidence, but to some extent to the lack of proper finance and credit machinery. Its existence, however, is a sharp deterrent to public works construction. It is particularly severe in rural areas where the absence of a developed credit system makes the borrowing of money almost impossible. In Haiti, for example, the effective rate of money lending to farmers may be 100 per cent or more. It is extremely doubtful whether much public works would or could be built even in the United States if interest rates varied from 20 to 100 per cent. The Planning Commission of India, in its first five-year plan issued in 1951, emphasized the fact that "The progressive substitutions of usury by organized credit at reasonable rates are also steps calculated to promote economic and social equality. The Commission recognized, however, the high importance of the relevance of tax evasion to all of its proposals."

That many of these defects have their roots in the recurrent instability of governments is confirmed in the comments by Wyman R. Stone (1952), Director of the Institute of Inter-American Affairs. He comments "I am afraid that one of the biggest problems is essentially that of the stability of governments and the lack of confidence that people with money have toward investing in the future of their own country." He further points out that in the evolution of many of these countries there is a long tradition of siphoning out of the country a good

deal of the money earned locally. The amount returned for local improvements was very small and the machinery provided for raising funds locally was virtually nonexistent. This historic picture accounts for the fact that in many instances the governments are largely central in character, with a minimum of local autonomy for development of local tax resources.

EXAMPLES OF FINANCING

It would appear, therefore, that in many of these countries resources are at hand; they are in general either kept out of circulation or are channeled into speculative and high interest bearing risks, or they move into investments outside of their own countries. Can these practices be readjusted and reversed? How may the local financing of public investment be strengthened?

In Latin America there are evidences that such trends may be reversed and that local financing can and may be increased. The experience of the International Bank is of special interest in this connection, because it exercises fairly rigid control over its own loans. It has developed a wide international experience and it attempts to subject its loans to the kind of scrutiny and criteria which would normally prevail in England or in our own country. They share the view that in many of the underdeveloped countries the amount of private savings is surprisingly high and compares not too unfavorably with the rate of private savings in the countries of Western Europe and in the United States. There are notable exampfes of this general pattern in Brazil, Mexico, Colombia, Chile and India, where active markets in corporate stocks have been developed.

The International Bank itself has been able to promote successfully a great deal of local financing in connection with its own loan operations. It has a long series of such local successes in India, in agricultural and power development; in Pakistan, in agriculture; in Thailand, in irrigation and other loans; in Iraq, in flood control projects; in Ethiopia, for highway and other development; in Southern Rhodesia, in electric power development; in Mexico, in light and power development; in Nicaragua, for highway construction; in El Salvador, for hydroelectric power; in Brazil, in the development of traction facilities and power; in Chile, for both power development and the exploration of underground water resources; in Turkey, for electric power, irrigation and flood control. These represent only examples of the directions in which these lending agencies have moved in order to strengthen the use of local fiscal resources and to increase their application to public improvements. Given time and imagination, coupled with an understanding of local financial difficulties, it is possible to develop in many of the underdeveloped countries the use of local resources to an extent hitherto unanticipated.

Without an understanding of the underlying problems of financing public works, the health official and the engineer are unlikely to make important progress in the installation of sanitary works in underdeveloped countries. They must master not only the basic difficulties in these fiscal structures but they must assist in so strengthening and orienting them that the key to the applica-

tion of their technological solutions to disease prevention will be more widely available.

That this essential feature of sanitary engineering practice is of extraordinary importance was recognized by the Expert Committee on Environmental Sanitation of the World Health Organization at its very first session in September, 1949. It stated in its Report to WHO that "WHO must make clear very early in its sanitation efforts that it is frequently cheaper to provide adequate and safe public water supply and sewerage facilities than to perpetuate individual private, costly and dangerous springs, wells, cisterns, cesspools and septic tanks. Both health officers and sanitary engineers on occasion lose sight of this important fact. This concept, however, can be applied only when ingenious but orthodox fiscal procedures and the use of local materials are carefully explored and adopted. The prevailing rates of interest payment are often more important features of fiscal programmes than are the amounts of capital investment. Excessive interest rates are common in many underdeveloped countries, where private risks may be very high. The pooling of loans, supported by more favourable governmental reservoirs of funds, may result in lower interest rates. These and other valid fiscal approaches need early study, recording and dissemination in countries requiring public facilities. Successful practices developed in financing, and they are numerous in many countries, should be the subject of a special monograph for wide distribution."

SUMMARY

General agreement may be presupposed upon the thesis that, in preventing disease and promoting health in the Tropics, the application of solutions lags behind scientific knowledge. One of the major obstacles to the more rapid introduction of sanitary facilities lies in the presumed deficiency of money. A recognition of this deficiency, coupled with a successful search for resources, give the best promise of more rapid accomplishments.

A number of recent studies of underdeveloped countries disclose larger amounts of fiscal resources than has been normally assumed to be at hand. Their failure to be moved into the public works market rests primarily upon absence of confidence in government, of modern safe financial institutions and of general public understanding. Because of these factors, the professional public health worker may well broaden his sphere of interest and influence to encompass these aspects of the society within which he works, if he hopes to speed up public health attainments.

It is increasingly apparent, as Balfour (1950) pointed out several years ago, that "public health programs must be coordinated with a general plan to develop all spheres of social activity, including education, agriculture, communications and industry." To this list, this paper adds that of the economics of public works expenditures!

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TROPICAL SANITATION IN ASIA—PROBLEMS AND PROSPECTS[1]

THE PUBLIC HEALTH PROBLEM IN TROPICAL AREAS

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The term "tropics" is interpreted to mean those regions which fit the cartographic definition, and also the other extensive warm and torrid regions of the world where life expectancies are very low. Together, these contain the great majority of the world's peoples. Although this great area contains salubrious communities, it is characterized by public health problems which are basic deterrents to socio -economic progress.

The three chief infections in this great area appear to be malaria, the filth-borne diseases and tuberculosis, although other infections may be paramount in some regions and in most regions there are many important public health problems. Of these three diseases, the first two are primarily amenable to environmental control measures, i.e., by the generic practice of sanitary engineering. Malaria is commonly considered to be the chief problem, and at the present time certainly is the most readily controllable of the three; but it is first in rank largely because the malarial infections are usually lumped together due to common mode of transmission and methods of control. However, when the salmonelloses and the shigelloses are combined with amebiasis and the excreta-borne helminthic infections under a filth-borne disease caption by virtue of their common, basic mode of transmission, the position of malaria is at least threatened.

Since the subject of malaria has been so ably covered at previous meetings of the parent societies and no doubt will continue to be well covered, I have chosen to concentrate mainly on the problem of the filth-borne diseases. Tropical Asia has been chosen as a focal point, but much of this discussion applies to other tropical areas as well.

BASIC SOCIO-ECONOMIC CHARACTERISTICS

From the point of view of the sanitary engineer, a key point in approaching the question of sanitation is that tropical Asiatic populations are essentially rural. Ancient but primitive agricultural practices prevail, practices which, in places, may result in very high productivity per acre but which are always featured by low productivity per farm worker. Since man cannot leave the land for the city unless the farm family raises a food surplus, or unless high industrial productivity permits the exchange of manufactured goods or extracted materials for regularly imported foodstuffs, the people of this great area are still pinned mainly to the land. Between 80 per cent and 90 per cent of the 400 odd million people of the

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Indian subcontinent, for example, are rural dwellers in spite of metropolitan centers such as Calcutta and Bombay, and it is in these rural areas that malaria and the filth-borne diseases are mainly concentrated. In contrast, only 36 per cent of the population of the United States lived outside of cities in 1950 and a large part of these were not engaged in farming. *Spelled out, the job is one of rural sanitation.* Moreover, there are no indications that sanitary improvements in cities have stimulated, or will stimulate, similar measures in isolated villages.

Those familiar with the rural landscape of this country with its scattered farmhouses and individual sanitary utilities might be inclined to write off filth-borne disease control in tropical Asia as a hopeless undertaking. But they would be overlooking another population attribute—that those people live almost entirely in compact villages. In India proper, for example, the rural population resides in an estimated five hundred thousand to seven hundred thousand villages with an average of around 500 persons per village. This means that public sanitation measures of a mass-benefit character, such as safe village water supplies, not only are warranted, but that the per capita cost generally should be lower than in cities due to the utilization of shallow ground water sources and no need for distribution systems and power-operated machinery.

PUBLIC SANITATION—A PRIORITY ACTIVITY IN TROPICAL AREAS

It may seem unnecessary to mention what seems self-evident to so many of us; but for the benefit of those living in temperate climates and protected environments, whose public health experience and training have been acquired under conditions grossly different from those which prevail in the tropics, the case for tropical sanitation cannot be restated too often.

Since the epidemiologic justification for tropical sanitation is well established, the sociologic case might be presented. The global history and evolution of public health programs and needs can be roughly divided into the following eras:

1. Era of introductory and early developing medical care, starting with the tribal witch doctor.
2. Era of public sanitation.
3. Era of personal hygiene, "sanitation as a way of life" and positive health.
4. Era of geriatrics.

The title of each era represents, not the totality of public health needs and indicated activities within the period, but rather the area of greatest need and dominant concentration of effort. With progression from one era to the next, the activities of antecedent periods are not abandoned. Rather, they are continued and maintained as entrenched functions at activity levels in proportion to degree of need. Conversely, the roots of each era originate in the preceding period.

At the present time the public health movement in the United States has reached the second half of the third era, i.e., personal hygiene and "sanitation as a way of life", with reconnaissance in force into the geriatric era. But in tropical Asia, no country has advanced in public health beyond the early stages of Era 2—public sanitation—and some clearly have not yet passed beyond the first.

The "four era" pattern of evolution in public health not only satisfies epi-

demiologic criteria but is further dictated by the general sociologic climate. In consequence, a balanced program of public health is not one which transplants the public health pattern of an Era 3 country into an Era 2 country, but rather one which fits the conditions of the country. More specifically, is it not a misapplication of effort to try to make people "health minded" in personal hygiene while they are living in community filth and are economically destitute as a result of community-wide malaria? Are not successful demonstrations in mass-benefit sanitation essential paths of approach toward public health education, and foundations for personal hygiene? General Hugh Cumming, a public health administrator of broad experience, gave a partial answer when he said: "I think the statement is true that a seriously malaria-ridden population is incapable unaided of maintaining a well-balanced health programme. The obvious indication is to get rid of malaria first, put the community on a par with others which fortunately do not suffer from this handicap, and then proceed with normal development in affairs of health and of economic and cultural development".

The public health movement in the United States is imbued with the concept of citizen participation, and from this point of view some types of tropical public sanitation projects appear weak. What is the answer?

In the first place, the public health profession in this country tends to have a major blind spot in its understanding of the total ecology of public health and socio-economic processes. By tradition, it has become divorced from the actual construction and operation of municipal sanitary utilities which support the socio-economic and public health structures of organized communities and has concentrated its attention largely on other activities which require personal health consciousness in one form or another.

Viewed from the broader perspective of general government, the true criterion becomes one of citizen interest or public support, of which citizen participation is a part. Within the past 50 years the highway movement over much of the United States has progressed from a small-scale citizen participation program in which each farmer was responsible for maintaining the public road passing by his property, to a service program of great magnitude, performed by local and state governments. Likewise, in this country city streets no longer are built and maintained, to any appreciable extent, by the direct labor of tax payers under the guise of the street tax. All of this was accomplished without organized propaganda by highway departments. There was a similar trend from individual to public water supplies, from privies to municipal sewerage systems, and from home garbage disposal to municipal collection systems. Can anyone maintain that public interest in and support of any of these have declined as a result of loss of personal participation? Has interest in personal health and hygiene dwindled as a result of these successful demonstrations in community cleanliness? Are not progress in, and support of, our third and fourth era activities in public health possible only because of the health consciousness and economic well-being generated by these contributions of the second era?

In the second place, community participation should be expected and obtained on most public sanitation projects in the tropical Eastern Hemisphere, including

those in rural villages. Money, materials and equipment contributions commonly are out of the question, but labor usually is available and should be utilized. Where proper supervision and training are supplied, all or most of the project labor required for DDT house spraying can be provided locally. This is likewise true of village water supply projects involving dug wells and comparable types of construction.

In any society, public sanitation projects owe a large measure of popularity to social values aside from their disease control benefits, and this is especially true during the pre-personal hygiene eras. It is for this reason that community sanitation is the Paul Revere of preventive medicine. Projects which directly and clearly benefit community comfort, convenience, or agricultural well-being serve this introductory purpose. DDT house spraying, village water supply and anti-malaria drainage projects are in this category.

In the third place, citizen participation can reasonably be expected in sanitation activities in the pre-health consciousness period wherever these confer other direct, tangible benefits and are of a character requiring personal action. Agricultural workers have shown that the technique of practical demonstration is successful in achieving the rapid adoption of improved agricultural practices by village farmers in communities where prevailing practices date back many centuries. Over most of Asia and in many other parts of the world, human and animal excrement are an essential plant food resource. The realization of safer practices in disposal on a broad scale is dependent now and in the forseeable future, not on burying human feces in holes in the ground, but on the development of simpler, safer methods of utilization which will also benefit agricultural production. The rural dweller will cooperate in this health objective whenever he can grow more crops by so doing, and whenever the agricultural benefits are made evident by local practical demonstration.

RURAL SANITATION NEEDS

There are two basic needs in rural sanitation for filth-borne disease control—provision of safe village water supplies and the safe disposal of human excreta. The approaches to, and solutions of, these problems are quite different. Mainly, they require separate consideration; but one point they have in common deserves passing attention, namely, sanitary standards.

The transmission of conventional water-borne diseases by *municipal* water supplies in this country is essentially nil, and has been for some time, thanks to the general competency of water works management, heavy investment in plant, unceasing surveillance by public health agencies, and a host of other contributing factors. Our objective today for these supplies is not the control of conventional water-borne diseases but the elimination of even remote hazards of transmission, and diverse other sanitary and public health considerations. Our concern is with refinements in protection, and our standards reflect this.

On a first assignment outside of this country in 1940, I was shocked, while making my first public water supply inspection, to find that the bacteriologic quality of the untreated surface water in the distribution system exceeded the

Raw Water Limit in use at that time in the United States, i.e., the water was too polluted for safe treatment by conventional filtration and chlorination processes. Since this is not an isolated instance, the public interest is served best under such circumstances by a program directed against gross sanitary defects. Design, costs, construction, and operation variously should be kept as low and as simple as possible. Minor hazards should be corrected only where there is no sacrifice of other objectives. This sounds like ordinary common sense, and that's all it is.

The same general principle applies to the disposal of human excreta, as will be discussed under that heading.

The provision of "relatively safe" sanitary facilities represents the principle of the calculated risk, well known to civil engineers as well as to the military. All engineering construction involves losses of human effort and resources expended in construction, and as an objective, the socio-economic value of the completed construction should exceed these losses by the widest possible margin. Today, in countries of limited resources the functioning of calculated risk planning in engineering construction is more evident than in this country, and the losses may at times include illness and death which could have been avoided at increased cost of construction.

In this connection, the civil engineer has been rightly criticized in many instances for the creation of man-made malaria conditions, where these are a consequence of the uncalculated risk, i.e., poor engineering. But the criticism has not been warranted in other projects where direct benefit to human welfare far outweighs loss, or threat of loss, and the cost of prevention is great, or additional project resources are unavailable. This is more frequently encountered in impoverished countries where the construction of roads, railroads and irrigation systems is vital in the race against famine.

VILLAGE WATER SUPPLIES

DDT house spraying in highly malarious areas has properly been given priority status in most national and international health programs in the general area. Malaria is a primary problem and the method of control by use of DDT is economical, relatively simple, generally yields quick results and possesses many ancillary values in disease control and public health education. Because drinking water is an intermediate mode of transmission for diseases spread by multiple routes, the sanitation of village water supplies possesses a somewhat lower order of public health value. On the other hand, the capitalized per capita cost of improved village water supplies should be much lower than that of DDT residual house spraying, and in addition ancillary socio-economic values loom large in importance, benefit is quickly realized in the community, the work has greater popular appeal, and construction generally is simple in character. Aside from diseases transmitted by drinking water, provision of village water supplies is also an important indirect method of schistosomiasis control.

Like DDT spraying, although village water supply sanitation can be quickly and economically accomplished in the individual community, the total need is so vast that only slow progress can be realized on the overall problem. Such

progress can be accelerated by a passion for simplification and a high order of resourcefulness on the part of the directing sanitary engineers. The true measure of caliber in the constructing civil engineer, and the sanitary engineer in rural sanitation, lies largely in the degree to which these qualities have been developed, and the sanitary engineer whose main interest is in progress had better stay at home.

For tropical areas in general, public health agencies characteristically have assumed responsibility for engineering programs of a small works nature, while public works authorities have performed heavy engineering construction. This pattern is considered entirely appropriate and is based on inherent differences in program philosophies. But public health agencies engaged in rural sanitation generally have failed to establish adequate linkage with agricultural programs, and there should be far greater realization that rural sanitation activities, properly simplified, are not only sanitary engineering functions, but are also agricultural engineering functions. The agriculturist occupies a unique position of leadership and confidence in the rural community and basic rural sanitation measures are properly within his sphere of interest. The sanitary engineer should make every effort to extend his services by fostering the interest and active participation of the agricultural field worker. This is especially important when it is realized that in a pre-health minded community, the motivating interest in improved water supply on the part of village leaders and citizens is concerned with occupational, convenience, and physiological benefits.

THE SAFER DISPOSAL OF HUMAN AND ANIMAL EXCRETA

The short cut from anus to mouth is caused variously by soil infestation and pollution including water-borne disease aspects, by flies, by unclean hands and by polluted food. Naturally, the relative importance of these modes varies from place to place. Avoidance of occupational defecation in croplands and of unclean hands are personal hygiene measures that probably will not be changed appreciably in these areas during our lifetimes. The present almost universal use of night soil and manure for crop fertilization in the general area is an economic necessity and present methods of storage and utilization are responsible for much house fly breeding, soil and surface water pollution, and most of the house fly infection and direct food pollution. The provision of sanitary pit privies and bored hole latrines has certain applicability where night soil practices are not employed, but these are limited in the rural areas.

The great need is for the further technical development of composting methods along joint public health-agricultural lines, and for the promotion of such measures after development as a basic part of the agricultural program. Present night soil utilization practices do not apply this plant food in its most assimilable form or conserve its full plant food value. The development of simple composting methods which will improve these values and concurrently greatly reduce disease transmission is theoretically feasible because aerobic nitrification processes work in the joint interests of plant food availability and pathogen destruction.

Those familiar with sanitary pit privies and bored hole latrines in actual usage

know that in protecting the public health, they are a far cry from water-carriage sewerage and the better types of municipal sewage treatment, yet few would question their great public health benefits. Similarly, the rational public health objectives of a composting program should not and cannot be the production of a completely safe material, but rather will be a major reduction in the frequency, extensiveness, and degree of human infection. Scattered studies indicate that composting may have inherent limitations in the destruction of some helminths and their ova. Unsatisfactory farm operating practices with even simple processes are inevitable.

PERSONNEL

In programs of technical aid from outside, what professional disciplines should be represented in the missions provided? Clearly, these are team operations, with all professions represented; but what is called for is a sanitation team, rather than a public health team as recognized in this country. Beyond this, there should be a fusion of health aud agricultural interests towards the objective of joint sanitation-agricultural teams.

The type of service rendered cannot be restricted to high-level advice, but must be focused on local demonstration projects, analogous to WHO malaria control and Point IV-FAO agricultural activities. In line with the sanitation era concept, is it not reasonable for approximately 80 per cent of the professional staffs to be composed of sanitarians of one type or another—medical, engineering, and related sanitation personnel, including malaria control and field training specialists? The remainder, representing other activities, should provide service in balance with social and public health needs and expediencies. Since local demonstrations in rural sanitation are contemplated, personnel experienced in this phase of public health is indicated and there is a particular need not only for sanitary engineers, medical sanitarians and entomologists, but also for individuals currently known in this country as sanitarians. A mission of this type is indicated for the typical nation, with a different type of group for less advanced areas, including those with nomad populations. A continental field research station in the technical development of composting would further require a variety of scientific disciplines. The furtherance of professional training at a few selected regional training centers would lie outside of the individual country program and likewise would require appropriate specialists.

SOME HUMAN FACTORS IN SANITATION PROGRAMS IN AFRICA[1]

J. W. WRIGHT

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The multitude of environmental sanitation problems which beset the continent of Africa are familiar to almost every student of tropical public health, as is, I believe, the vast store of knowledge available today on how many of these might be resolved. What may not be so generally known, however, are the factors hampering the full application of this knowledge, and I propose during the time at my disposal to outline some of the more important of these.

Probably one of the most significant of these, through circumstances beyond his control, is the African himself. The peoples of Africa—and here I am referring to the Africa south of the Sahara, excluding to a large extent the far more developed Union of South Africa and some coastal areas that have been in contact with the outside world for over a century—are at the present time undergoing a change of a rapidity unprecedented in their history. No phase of their lives remains unaffected and they are frequently exposed to stresses and strains with which they are poorly equipped to cope. The impact of European culture is, in the area under discussion, in most cases not more than 60 to 80 years old and is now exerting a destructive effect on their tribal life and the Africans are being forced to adapt themselves, as best they can, to unfamiliar and alien conditions. Their problem is twofold: adjustment to the abandonment of their traditional social structure with, at the same time, an urgent necessity to catch up with a mode of life essentially fluid in character and difficult enough for those who conceived, or at least fostered it.

To appreciate this fully it is important to have some understanding of the cultural background of the primitive people of Africa. One of the clearest descriptions of African tribal life has been given by Carothers and one cannot do better than quote from it:

"The primitive African is governed by a large, complex and rigid social organization based on a wide extension of the family system, and by which a high degree of security for the individual is achieved in face of the manifold hazards of nature. This organization implies and demands the observance of meticulous rules and restraints in regard to manners and behaviour in all departments and at each stage of life. These rules, although unwritten, are so well-known that any infringement implies and is recognised as having an unsocial motive. There is no room for free thought nor for misfits and even secretive, solitary or outstandingly successful persons are suspect.

Everyone sooner or later knows the whole culture of the group; there is little

[1] One of four papers composing a Symposium on Sanitary Engineering in the Tropics held on November 15, 1952, during the Annual Meeting of the American Society of Tropical Medicine and Hygiene at Galveston, Texas.

specialization and a great equality of knowledge. Status depends on age rather than on intellect, knowledge or achievement. Occupations and rules of conduct are strictly arranged and the African constantly acquires more rights and duties in an ever-widening field as the social organism of which he is an essential part increases in size with each rise of status.

In general the primitive African is very close to nature and the realities of life and death. Disease and misfortune are familiar experiences and come unaccountably like bolts from the blue. He feels that whatever stability and strength he has comes from the fact that he is part of a larger organism and that he can only preserve this modicum of power by playing most meticulously his special part in this organism. This attitude governs his whole life and results in extreme conformity to tradition, conservatism in thought and fear of innovation. In the absence of any general conception of the inherent nature of cause and effect, he regards the workings of fate as arbitrary and haphazard and, provided he has obeyed the traditional rules, he rarely blames himself for his failures or misfortunes, but is apt to attribute them to the anger of some external agency and believes himself bewitched. Since his rules of behaviour only apply within his group, and in relation to his clan land, and since he has no abstract codes, he is left without a guiding star when he leaves his tribal sphere. Finally in the absence of children's toys and a written language, the African is largely dependent for his amusement and his learning on the spoken word".

The influence of the outside world has brought great changes to the African tribes. The call of the gold, copper and diamond mines and of the industries of the many rapidly growing towns, for more and more labor has encouraged the able bodied men voluntarily to leave their tribes. Often as many as half the men are away from their villages at one time, disrupting completely social and agricultural life. Yet because this absence is not permanent there has not been the large-scale constitution of African societies in and around industrial centers in which acceptable family life could be established. The greater part of African life is therefore still based on the tribal tradition, but burdened with the economic responsibility of modern life.

Many Africans do break away completely from their tribes and attempt to make their homes permanently in the urban areas. The problems which confront these detribalized Africans are considerable. They are forced to establish a new set of standards to replace those to which they have been accustomed. They find the ways of urban life bewildering and often incomprehensible, and even in accepting them do so blindly and without complete understanding. In emergency they fall back instinctively on their traditional teaching and so find themselves in a state of continual mental conflict.

Against this complex background it becomes clear that the success of programs of social, health or other development will depend largely on the degree of understanding with which the basic elements are presented to the African. In no field will this be more important than in that of environmental sanitation which, striking at the very root of his existence, whether in the tribe or in the rural or urban community in which he has made his life, may be most difficult for him to

accept. Precipitate action must be avoided at all costs and a step-by-step approach developed. Yet the pressing fact remains that the future development of the continent may well depend to a large extent on the success achieved in raising environmental sanitation standards.

It becomes clear, even after a cursory study of the problem, that education is the key to its solution and the question immediately arises, are the educational facilities available in Africa adequate to meet this challenge? It is unfortunate that the answer must be in the negative for the present, although millions of dollars are being spent each year on improving the situation.

Education in Africa has traditionally rested with the Missions, whose efforts have been, of necessity, limited by their resources. It is only comparatively recently that Governments, awakening to the gravity of the problem, have given any substantial assistance. But even with this help there are still too few children attending school for too short a time. In a recent survey of school facilities in British territories in Africa it was revealed that the proportion of children attending school ranged from 2 to 14 per cent of the total African population, with the average between 7 and 8 per cent. The situation in the other territories of Africa is comparable. These figures in themselves do not convey the true gravity of the situation. Not only is there a very high absentee rate partly because of sickness, but far too many children leave school only after three or four years tuition. In one country of Africa it has been recorded that of about 170,000 children attending schools of all types in 1943 only 42 successfully completed the full primary syllabus. The basic factor here is economic. In some cases schooling is not free and the fees, low as they are by Western standards, frequently represent an overwhelming burden to the parent. In other cases although no charge is made the distance between home and school often makes attendance erratic.

This tendency of the child to leave school before a reasonable standard of education has been achieved has a long term effect on the quality of schooling generally. An number of insufficient pupils qualify annually to continue wita courses of teacher training, the result being that education today lies largely in the hands of persons not qualified according to Western standards. In one territory, for instance, in 1942 only 5 per cent of the teachers in unassisted schools were properly qualified. One of the consequences of this short and unsatisfactory education is that the child goes into the world without sufficient training to cope with the new life but with enough to bring dissatisfaction with tribal conditions.

The life of any child at school is not made any easier by the fact that he or she is seldom able to put into practice in the home what is taught in the school. Lessons in hygiene are lost in an environment where the parents are illiterate and ignorant and frequently the new outlook of the child causes friction in the family.

Many governments have made efforts to assess what would be required to put education in their territories on a sound footing, but in all cases this has been found to be beyond their economic means. In one instance it was found that, assuming unlimited funds were available, it would take almost 50 years to provide every child of school age with an adequate primary school education.

These facts plus the inescapable importance of adult illiteracy, bring home with great emphasis that immediate and large-scale action is required if the emergency is to be met. To many students of Africa the only solution appears to lie in the mass education of whole African communities, from which the will to accept and understand change may grow.

The problems associated with the development of such programs are admittedly immense but if the complications inherent in a process of technical evolution in which development is outstripping education are to be avoided, there appears to be no alternative. A start has already been made in many parts of the Continent, but success will depend to a large extent upon the growth of enthusiasm in the African for his own advancement and the part the educated African may be willing to play in helping his less fortunate brothers. The spark which has made similar programs successful in other countries has so far been lacking in Africa, and until it has been kindled it would be unrealistic to hope for any dramatic success in the development of sanitation programs, in which permanent change of the environment and the fostering of self help are the primary objectives. In the light of these facts, what is the status of environmental sanitation in Africa today?

Some progress has been made in many parts of Africa during the past few decades in developing acceptable standards of environmental sanitation. In some of the towns and cities of West, Central and East Africa and of the Union of South Africa high levels have been attained that compare favorably with the best in Europe. In addition many programs of vector borne disease control have been undertaken in almost every territory of the Continent with considerable economic benefit to the communities concerned. Amongst these may be mentioned the programs for control of trypanosomiasis, yellow fever, smallpox, bilharzia, plague and malaria. Since the Malaria Conference in Equatorial Africa sponsored by the World Health Organization in 1950, efforts to control the latter disease have been intensified. The Governments of Liberia, French Equatorial Africa and the French Cameroons have, for example, with the assistance of WHO and UNICEF, initiated programs which will form the foundation on which truly national control of the disease will be based.

In rural sanitation, however, the collaboration of the individual is vital and only from this can real lasting results be attained. No more than the foundations are being laid at the present in this field of sanitation but what is being achieved on a relatively limited scale is sound. The Anchau Resettlement Project in Nigeria is, for example, almost a classic in rural sanitation practice and represents a good foundation for future planning. Even in this preliminary work the authorities are being handicapped by the lack of adequately trained personnel. In the light of the educational conditions throughout the Continent this is not surprising. Only in an improvement of these conditions will the answer to this be found.

The structure and staffing of sanitation organizations has in almost all non-self-governing territories been based on a compromise between the traditional system of the metropolitan country and the demands of local circumstances.

The planning and overall supervision of environmental sanitation programs are performed invariably by a relatively small number of Europeans with European training, the responsibility for field implementation resting largely with African personnel. The adequacy and type of training of the latter vary from territory to territory.

The Public Health Engineer as recognized in America is virtually unknown in the greater part of the Continent, and it does not appear that, with the limited university facilities available, this situation is likely to alter greatly in the near future. The only university in East Africa, for instance, serves a population of approximately 14 million people. Its output of teachers and of medical, agricultural and veterinary graduates is limited to only 40 per year. Sanitation programs will in future be built up around the sanitarians, the majority of whom will be recruited from the local population and trained in National or Provincial Schools of Hygiene. The type of training being developed for the African sanitarian and the way in which he is being used are interesting. This is briefly the situation as I saw it in Southern Rhodesia and Uganda recently.

In Southern Rhodesia a government school has been established in Domboshawa, near Salisbury. This school was originally established to teach Africans trades and farming, but a hygiene branch for training what are called "health demonstrators" has recently been added. This concept of teaching the various trades and disciplines together has a number of advantages, one of the most important being that the students learn each other's problems and avoid too narrow an approach to any one specialty. Students receive a comprehensive 3 years' course on the theory and practice of hygiene and sanitation and building construction. On qualifying, they are sent in pairs to live and work in the rural districts where they not only teach the basic principles of environmental sanitation, but demonstrate how these may be put into practice. Their work is under the technical guidance of the European sanitarians and is integrated with other health functions.

In Uganda a national plan has been devised which will in time provide a trained sanitation worker for virtually every part of the country. Four types of personnel are involved: first, the European sanitarian, technically responsible for an administrative district, of which there are 14 in the territory. Secondly, there is the African Assistant Sanitary Inspector, in charge of an area with a population of about 60,000 persons. This inspector has a good basic education and three years' training in the Government School of Hygiene (which is at present capable of training about 12 students annually). The inspector will have up to 12 hygiene orderlies under his direction. This third group has a lower standard of basic education than the inspector, and only one year's training at the School of Hygiene. Finally, there is the health orderly at the village level who is drawn from the community and trained in the field.

The authorities in Uganda and Southern Rhodesia would be the first to admit the limitations of their plans. They are hampered by lack of funds and the difficulty in obtaining the right type of student and also reliable workers in the community.

In Liberia the WHO has started a training scheme for sanitarians and the first group of pupils qualifying under the plan will soon be in the field. Plans are now under consideration for an extension of the training scheme in collaboration with the Booker Washington Institute. It will, however, be many years before the demand for staff is met.

These plans may appear inadequate and imperfect by the standards of the Western world, but they represent, in fact, great progress and have been made possible only by the enthusiasm, patience and understanding of the persons behind them.

Where does the future of environmental sanitation in Africa lie? It may be summed up under four headings:

(1) The continuation and expansion by Governments of programs for control of insect-borne disease, in which the degree of collaboration required from the community is not too demanding and yet from which considerable and immediate returns may be expected. In these, every opportunity should be taken to bring home lessons of hygiene to the people in the villages and rural districts.

(2) The development of rural sanitation programs on as wide a front as possible. An important part of these programs might be the establishment of local research units to study the best and cheapest ways to carry out simple sanitation works with local labor and materials.

(3) Improvement in the quantity and quality of training facilities for environmental sanitation personnel at all levels throughout the Continent with emphasis throughout the training period on health education, and the organization of sound health structures in which they may be used.

(4) Encouragement of the development of mass education schemes for adults and children throughout Africa, and collaboration in these to the greatest possible extent by environmental sanitation organizations.

(5) A continued study of the African and his ever-changing cultural status, so that sanitation programs may at all times be planned to keep pace with development. Precipitate action of any kind must be avoided.

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THE ROLE OF THE MECHANICAL ENGINEER IN PUBLIC HEALTH[1]

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In recent years, the field of public health has experienced a great influx of specialists. Prior to 1910, medicine and nursing were almost the sole professions within the health department. At that time medicine was almost entirely clinical. As preventive medicine developed it was imperative to enlist the services of many scientific disciplines in a cooperative attack upon the complex problems of modern public health. Thus we now find that the dentist, the sanitary engineer, the bacteriologist, the mycologist, the virologist, the physicist and may others have a definite place in the field of preventive medicine.

As each of these professions has increased its activity in research and field operations for the control and prevention of disease, it has had to reach for new tools with which to perform its task. Today an examination will show that a surprising proportion of these tools are not biological, statistical or diagnostic in nature but are instead purely physical tools, apparatus and equipment. Today, who could envisage a modern health department without x-ray apparatus, autoclaves, scales, forceps, hypodermic needles, refrigeration, examination tables, microscopes, animal traps, insecticide sprayers and countless other items which contribute to its efficiency? Similarly, what modern public health research project could made progress without drawing upon the resources of a scientific supply organization, an enterprise whose catalog is a veritable encyclopedia of the physical requirements of research? The modern concept of public health does not limit the field to the health department. When we consider the hospitals and the vast network of sanitary services protecting the modern city, the list of complex mechanical equipment is enormously lengthened.

Having become accustomed in our daily lives to the assistance of mechanical appliances, we frequently lose sight of the contribution of specialized physical equipment to the solution of problems in public health. With the realization of this dependence upon mechanical aids, we must in turn give credit to a hitherto largely anonymous benefactor in public health, the mechanical engineer. His is a profession trained in the design and production of mechanical equipment, in the operation of mechanical processes and in the attainment of maximum efficiency in the work of men and machines.

This is in contrast to the sanitary engineer whose work with the problems of air, water, food and shelter has long been recognized. The sanitary and the mechanical engineer may have a common goal, the betterment of public health, but they utilize different avenues of approach. The sanitary engineer works and thinks

[1] One of four papers composing a Symposium on Sanitary Engineering in the Tropics held on November 15, 1952, during the Annual Meeting of the American Society of Tropical Medicine and Hygiene at Galveston, Texas.

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largely in terms of the utilization of processes, labor and equipment to accomplish his goals. The mechanical engineer, on the other hand, is concerned primarily with the design, development and production of the equipment and tools required. He is the engineer you would call upon to design a new insecticide sprayer, a laboratory shaking machine, an autoclave, an automatic injection apparatus, a garbage grinder, an ultracentrifuge or similar devices. The design, production and testing of equipment such as this is the activity in which the mechanical engineer can and has made his contribution. We need not expect to find a mechanical engineer in every local health department, but today there is recognized an increasing need for men trained in mechanical engineering, with a background of public health experience. Such experience is required in order to interpret the needs of public health for industry and to produce the specialized apparatus for public health research. Today there is probably no such engineer on the payroll of any state health department. However, the World Health Organization has recognized the need for this discipline by appointing several mechanical engineers as expert panel members. The Expert Panel on Insecticides includes several such experts. Mechanical engineers were not eligible for the Regular Corps of the Public Health Service prior to 1947. However, there are at present throughout the world a few mechanical engineers employed in various governments at the federal level and the number is increasing. Nevertheless, the greatest contributions of the mechanical engineer to public health will continue to be made in industry, but his integration into major health units is increasingly indicated since it is here his talent can be employed to design specialized apparatus and equipment for specific operational and research programs for which tools are not available.

It should be borne in mind that these contributions are not limited to highly industrialized countries, for mechanical equipment is frequently much more vital to sanitation and other public health activities in under-developed areas than in those that are more advanced. In each case, but particularly when equipment is to be used in remote areas, careful engineering is required to assure durability, simplicity of design and maintenance, and low cost. Now let us examine in more detail a few of the activities and accomplishments of the mechanical engineer in this field.

For many years the mechanical engineer has been actively engaged in industries which are noted for polluting the air with smoke, tarry products and corrosive gases that in turn may menace the public health. An increased awareness of responsibility for this problem is now apparent among the mechanical engineers. Similarly, the exhausts of our millions of internal combustion engines have contributed to air pollution in metropolitan centers. Improved combustion design by the mechanical engineer can reduce this problem and has done so. A long step in this direction appears to have been taken with the recent report of the development of equipment which completes the combustion of fuel components normally wasted up the stack.

For many years, in these same plants, a war for survival has been waged between men and machines. During the industrial revolution the need for safety

in factories was hardly recognized, but since that time the mechanical engineer has taken the lead in the correction of unsafe situations, in building safety into machines and in training for safety.

In the production of optimum artificial environments the mechanical engineer has taken a leading role and, in so doing, has exerted an influence on the fringe factors affecting the total physical well-being of man. Further research on these problems is under way in an environmental laboratory in which almost any artificial environment can be established and its influence on man evaluated.

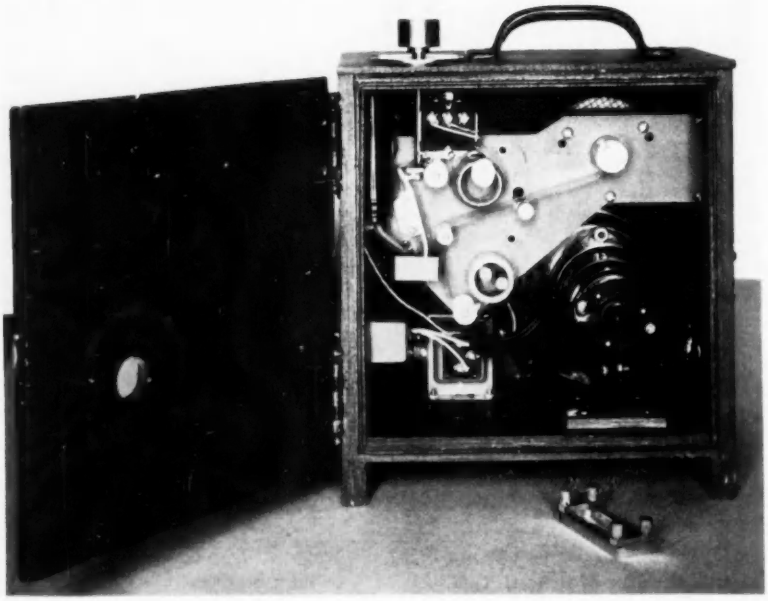

FIG. 1. A continuous recording dust sampler. Particulates in the air are impinged on the adhesive side of scotch tape, the tape moves through a staining bath and then meets a facing tape. The tapes, with the particles sandwiched between them, are stretched in a frame for examination under a microscope.

Preventive medicine, in its increasingly effective fight to control the vectors of disease, has leaned heavily on the mechanical engineer for tools to accomplish the end result. From the initial use of kerosene for the control of mosquitoes to the present day application of residual insecticides, the equipment for the application of vector poisons has been of crucial importance. In the early days of mosquito control, knapsack and compression sprayers for the application of oil and dusters for Paris green, were borrowed from agricultural designs. With the advent of the use of residual insecticides on the walls of sleeping rooms, there developed a need for specialized spraying equipment to implement the new tech-

niques. Designs were evolved in step with the development of new methods for the use of DDT and related insecticides until many of the requirements were crystallized by the World Health Organization's Expert Committee on Insecticides in establishing specifications for hand, compression, knapsack, and stirrup-pump type sprayers, hand dusters, chemical resistant materials, cut-off valves and hose connections. Mechanical engineers in public health are working closely with the manufacturers of spraying equipment to bring their products into compliance with the specifications.

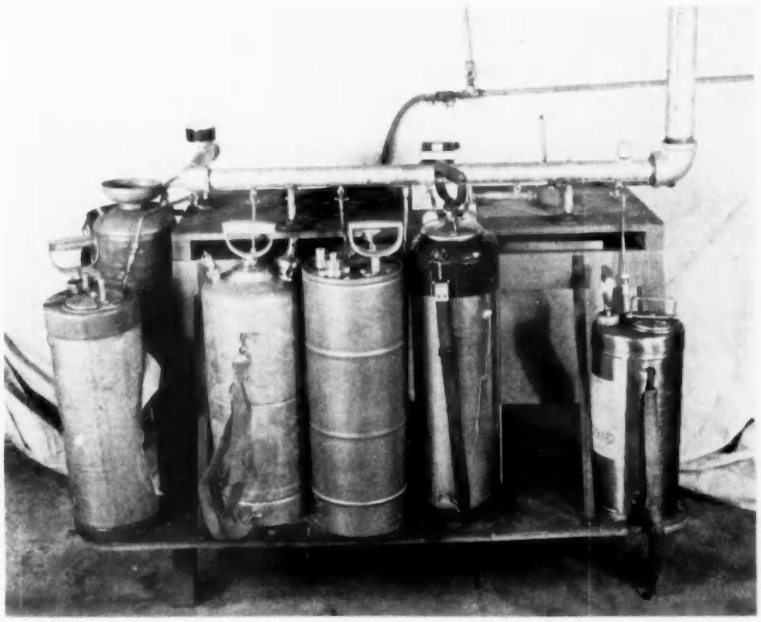

FIG. 2. A fatigue testing apparatus for compression sprayer tanks. To determine weak points in the tanks pressure is applied and released until 7,500 cycles have been accomplished or until failure results.

Currently, improvements in application equipment are moving forward. A constant delivery sprayer was introduced in 1948 and as a result of the recognition of its advantages commercial models are now being developed and produced in Italy, France, and the United States.

Application of the principles of mechanical engineering to the production of specialized research equipment has proved highly advantageous to the Technical Development Branch of the Communicable Disease Center in Savannah, Georgia. There the Equipment Development Section, staffed largely by mechanical engineers, has produced animal exposure chambers, electronic respirometers and metabolism rockers for studies on the toxicology of economic poisons. For re-

search on insecticides, a device has been produced which semi-automatically cements short threads to the backs of live flies for identification purposes; another has been constructed which automatically records the time of death and the number of flies killed by a formulation under test. For investigation in the field of air-borne pathogens, continuous recording dust samplers have been built (Figure 1), air flow meters have been improved and the relative efficiencies of a number of air samplers have been determined.

Contributions have also been made to the efficiency of field operations by a study of the ratproofing characteristics of various construction materials, by the improvement of rat guards, by the design of a new type of aerosol generator, by improved systems for the dispersal of insecticides by aircraft and by the testing of insecticide sprayers (Figure 2). This list by no means exhausts the specific contributions made by the Equipment Development Section. The list could be expanded indefinitely if one were to consider the contributions of the mechanical engineer to other fields of environmental health protection including water supply, sewerage, sewage treatment and the collection and disposal of solid wastes.

Thus the mechanical engineer is contributing his small bit to the public health. He does not ask to supplant any of the well known scientific disciplines now engaged in the field, nor for more renown, but he does ask that, when advantageous, his particular training and capabilities be utilized in the solution of problems important to the public health.

HEALTH ASPECTS OF THE COMMUNITY DEVELOPMENT PROJECT, RURAL AREA, TURRIALBA, COSTA RICA, 1948–51[1]

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Most human societies today give prominent emphasis to health. Communities recognize this basic need by establishing health systems which range from the folk practitioner in the simpler communities to a complicated interdependence of hospital, clinic, private physicians, dentists, laboratories and pharmacies in the more evolved communities. Little is known, however, of the factors which affect the rate of evolution from the simpler to the more elaborate systems, especially in tropical and subtropical areas, and how this process contributes to integrated community development. Neither do we know how and to what extent health influences community activities and organization. Nevertheless, very few scientists would question the validity of including health as one of the important aspects of the integrated approach required in a community development program.

As the solution of community problems seems to be at the root of the current efforts to improve the lot of mankind, further basic research on the role played by health in the economic, social and cultural status of a community must be conducted before we can approach with confidence the more complex problem of development for broader geographical areas. This paper describes the design and initial progress of a research project of this type in an area in which many problems of tropical medicine and hygiene are encountered.

Research design. In 1947 the Inter-American Institute of Agricultural Sciences

[1] The project was initiated by Julio O. Morales who supervised the collection and tabulation of the social, economic and agricultural data and Charles P. Loomis who helped in the design and execution of part of the sociological studies. They were assisted by Antonio Arce and Edwin Murillo. Marta Coll Camalez designed the health questionnaire and completed the initial collection and analysis of the data. The dietary surveys were designed and completed by Ana T. Blanco de Sariola and calculated by Margaret Moen Fiester.

The clinical studies were initiated by Dr. Nevin S. Scrimshaw and carried out by the following personnel under the direction of Dr. Oscar Vargas M., Director of Public Health of Costa Rica: Dr. Alfonso Salazar B., special nutritional examinations; Drs. Rodrigo Portocarrero and Rodolfo Vinocour, general physical examinations; Dr. Carlos Manuel Ortega P., dental studies; Dr. Lisímaco Leiva and Dr. Juan Dávila R., fluoroscopic examinations; Carlos Brenes Zamora, parasitological examinations; Fernando Leal, hematology and serum protein determinations; Joaquín B. Soto, serological and liver function tests and a supporting group of nurses and social workers. Dr. Emilio Picón assisted in the clinical nutrition examinations. Roberto Sasso was in charge of statistical analysis. Preparation of the final data was aided by a grant from the W. K. Kellogg Foundation.

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began economic and sociological studies in Turrialba, Costa Rica. A complete agricultural and population census was taken, and detailed maps of land use, land tenure, land ownership, and house distribution prepared. A sociological survey administered to 519 rural families was designed to discover informal prestige and clique leaders, community and neighborhood affiliation, characteristics associated with socio-economic status, and other fundamental social characteristics of the population.

Although a number of sociometric questions were included in the sociological survey, two proved sufficient to determine leadership status for the purposes of this study. "What families do you visit most often?" was used to establish clique groups and their leaders. "Whom would you select to represent you and the people of your area on a commission?" determined informal prestige groups and their leaders. The family or person mentioned most often within each informal group was considered the leader. Less than half of the rural families were included in this survey but they provided sufficient information regarding

TABLE 1

Composition of the sample by occupational groups and by type of subsample, Rural Zone, Turrialba, Costa Rica—1948

OCCUPATIONAL GROUP	NUMBER OF FAMILIES		TOTAL
	Purposive subsample	Random subsample	
Farmer	17	17	34
Laborer	16	73	89
Administrative personnel	5	3	8
Other	3	6	9
Total	41	99	140

the 25 neighborhoods surrounding the town of Turrialba to permit the determination of these groups and their leaders in the 1,241 families of this rural zone. Of the 132 "leader" families thus selected 41 were considered of major importance to their communities and were included as a group in the health study. This was done because it was felt that progress might be most rapidly brought about in the area by working with the leaders and that a thorough knowledge of their habits and attitudes would be essential for such efforts.

The remaining less important "leader" families were grouped with the "follower" families and a randomized sub-sample of 99 selected. This was stratified by neighborhoods in proportion to the families in each neighborhood so as to assure the broadest possible geographic distribution of the subsample within the area. This sample was of course necessary to characterize the community as a whole. Thus 140 families in all, 41 of the most important leaders and 99 from the remainder of the population were included in the health study.[2]

[2] Due to the nature of the sample, estimates of the population are calculated by multiplying the frequency distribution obtained in the random sample by 12 and adding the fre-

The major characteristic of stratification by neighborhoods concerns economic organization. As a higher proportion of the population of the community live in the "haciendas" than in the "small and medium farm" neighborhoods, the sample has 81 families in "haciendas" and only 59 in the other types of neighborhood. Usually the "haciendas" form both economic and social units and their boundaries coincide with the boundaries of the neighborhood. Both subsamples are divided in similar proportions between these two types of neighborhoods.

The health studies were initiated in cooperation with Michigan State College by administering a questionnaire to the 140 family subsample, to obtain information on: a) sanitary conditions of the home and surroundings; b) health and hygiene habits; c) available clinical, hospital, medical and dental facilities; d) dental care, and e) maternal and child care. Besides obtaining information on the conditions prevalent at the time of the interview, efforts were made to secure data on the opinions held by the families in relation to these conditions.

The 1948 health survey was followed in 1950 by a dietary study on the same subsample. The questionnaire covered: a) one-week and one-day dietary intake; b) seasonal consumption of foods; c) refusal of food and special food intake by individuals in the family; d) the knowledge of cooking and nutrition of the female head of the family; e) basic dietary problems recognized by the informants; f) composition of the family by age, sex and occupation; g) home production of food, and h) other general information. A total of 137 families gave this information.

In 1951, in cooperation with the Costa Rican Ministry of Health and the Institute of Nutrition of Central America and Panama, the same 137 families, making a total of about 900 persons, were given the following examinations; a) general physical; b) nutritional; c) dental; d) parasitological; e) fluoroscopic; f) hematological and serological; g) protein and vitamin serum levels; and brief medical histories were obtained.

The accumulation of these data on one community presents an unusual opportunity to study the interrelationships of health with social factors such as informal leadership and social class; with economic factors such as occupation and income; with institutional factors such as health systems and with the factors affecting community development. Furthermore, the Turrialba Community is expanding and changing its health systems under the impetus of the cooperative efforts concentrated on the Community.

In the presentation which follows it is not possible to discuss more than a few of these interrelations and the technical data cannot be presented in detail. They will be described and discussed in a series of specialized articles. This introductory paper is intended to explain the design and scope of the project and to discuss the possible applications of some of the more significant relationships thus far observed in a continuing community development project.

Adequacy of the diets. The weekly family diets in all groups were usually found

quencies obtained in the purposive sample. This procedure acknowledges the fact that each family in the random sample represents 12 families in the population, while the purposive sample was supposed to be a full-coverage of the "important informal leader" stratum.

to be deficient in vitamin A and riboflavin and frequently also in protein of adequate quality, calcium and ascorbic acid. Two-thirds of the families had no meat on the day before the dietary interview and nearly one-fourth used no milk at all. Although, 72 per cent were unaware of the unsatisfactory nature of their own diets, 90 per cent recognized that there was a relation between being well fed and being healthy. The magnitude of the education work necessary is revealed, however, by the fact that only about half of the people had heard of vitamins and the majority of these had little or no understanding of their role in nutrition.

TABLE 2

Differences in intake per consumption unit among economic groups, Community Development Project, Rural Zone, Turrialba, Costa Rica, 1950*

ECONOMIC GROUP	LABORER WITHOUT LAND	LABORER WITH LAND	SMALL FARMER	FOREMAN, STOREKEEPER, SKILLED WORKER
Calories	3213	3483	3753	3402
Protein, gms	78	86	88	79
Animal protein, gms	14	20	23	26
Calcium, gms	.4	.6	.8	.5
Iron, mgs	24	25	26	20
Vitamin A† "activity" I.U.	600	960	1230	1320
Thiamine, mgs	2.0	2.1	2.4	1.7
Riboflavin, mgs	1.0	1.2	1.3	1.2
Niacin, mgs	15	15	17	15
Ascorbic acid, mgs	67	79	106	112

* Intake is given as per consumption unit using as a basis N.R.C. (U. S.) standards for a physically active man except in the case of calories where the F.A.O. calorie recommendations are followed. Thus, for calories, age, weight and mean annual temperature are taken into consideration in determining the caloric needs of the family and a physically active man of the average age and weight of the adult males in the sample is used as a unit of comparison. The N.R.C. tables make no direct allowance for differences in weight or differences in requirement within adult age groups. Therefore, the reference standard for the calculations may be too high, since the people of Turrialba are on the whole smaller and lighter than the U. S. population. For comparisons among groups this is not important, but it should be taken into consideration in evaluating the adequacies of the group diets in respect to individual nutrients.

† Based on a recommended allowance of 3,000 I.U. of vitamin A. (The allowance would be 5,000 I.U. if two-thirds of the vitamin A activity came from vegetable sources, and 6,000 if all came from vegetable sources).

Eighty-one per cent stated that they would not be willing to accept skim milk, either fluid or dried, most of them believing that it had little food value. The average figures for calcium were found to be misleading because the 37 per cent of the families using calcium oxide in the preparation of corn for tortillas compensated for the remainder using wood ash and having a very low calcium intake. The fat intakes were very low, so that only 13 per cent of the total calories were derived from this source.

The diets were found to vary among the major economic groups as shown in Table 2. The differences in animal protein and vitamin A are highly significant

statistically. The slightly better diet for the families of workers with land to cultivate and the small farmer working his own land indicates that nutrition tends to be better when the family can produce part of its own food. The foods thus produced frequently include those which supply nutrients commonly deficient, a fact which emphasizes the importance of encouraging the rural family to raise part of its own food.

Although the specific data are not presented here, adequacy of diet also appeared related to leadership status. The families headed by a person who was both a prestige and a clique leader tended to have the best diets. These families were also better off economically, so that their superior diets may be partly a reflection of their superior purchasing power. Again the differences occurred primarily in their intake of animal protein and vitamin A.

In general the families living on large haciendas were not as well nourished as those from the medium and small farms, but this group included a larger proportion of workers without land to cultivate. As might be expected, family size affected the availability of animal protein and riboflavin to a significant degree, the larger families having less available per consumption unit.

Incidence of parasites. It is probable that the adequacy of the diet is further reduced by an adverse effect of multiple parasite infestation on digestion and absorption as well as by the physical drain resulting from several of the common species. Not only are the individual intestinal parasites found with the high frequencies listed in Table 3, but also 90 per cent of the people have two or more parasites, 56 per cent three or more and 17 per cent four or more. It is therefore not surprising to find that 35 per cent of the families have unsafe toilet facilities or none at all. Although 63 per cent of the families had privies, the majority of these were in very poor condition. The percentage wearing shoes is very small, 4.5 per cent in families with sanitary facilities and only 0.8 per cent in those without. Similarly the control of intestinal infestations and enteric diseases is greatly complicated by the uncertain nature of the wate supply. Only 14 per cent of the families obtained water from a spring at its source and 44 per cent used water from a river, pond or ditch.

There was evidence of awareness of these problems since 43 per cent of the 140 families in the sample were concerned about their water supply, 46 per cent about their sanitary facilities, and 13 per cent about their washing and bathing arrangements. At the present time malaria is only a minor public health problem. Most of the infections are now acquired during work on the coast rather than contracted in Turrialba. In the entire group four per cent of the people had positive blood smears for malaria and 3.7 per cent had palpable spleens.

Differences in the incidence of parasitic infestation between males and females were not significant, but the incidence increased with age in both sexes up to ten years and remained high thereafter. The tapeworm, *Hymenolepis nana*, was found more frequently in children under ten who showed an incidence of 7.5 per cent of this parasite. Protozoa were found slightly more often in persons from large *fincas* than from medium and small ones but the differences were not great. In the case of helminth and larval infections the differences were small and in-

consistent. As shown in Table 3, *E. histolytica* occurred more frequently in the laboring groups having their own land, but the differences among occupational groups in respect to the incidence of other parasites have very little significance. Except for *E. histolytica* which tended to occur less frequently in clique and prestige leaders, leadership status appeared to bear little relation to parasite infestation.

Although the percentage of persons having parasite infestations was found to be high, it was not practical to determine the intensity of the involvement. Annual treatments by the Ministry of Health may have kept extremely heavy con-

TABLE 3

Per cent incidence of intestinal parasites in 140 families, Community Development Project, Rural Zone, Turrialba, Costa Rica—1951*

	LABORER WITHOUT LAND	LABORER WITH LAND	SMALL FARMER	FOREMAN, STOREKEEPER, SKILLED WORKER
Number of persons	299	167	237	79
Endamoeba histolytica	18.7%	26.9%	9.3%	17.7%
Endamoeba coli	4.0	5.3	5.5	5.1
Giardia intestinalis	3.7	4.2	2.1	2.5
Trichomonas intestinalis	8.7	9.5	4.2	5.1
Chilomastix mesnili	5.3	4.8	2.5	3.8
Balantidium coli	0.7	0.0	0.8	0.0
Positive for one or more protozoa	28.8	39.5	18.6	26.6
Ascaris lumbricoides	58.5	58.7	62.0	70.9
Trichuris trichiura	96.3	94.0	96.2	94.9
Necator americanus	64.2	58.1	63.3	57.0
Strongyloides stercoralis	8.4	10.2	3.8	8.9
Hymenolepis nana	4.0	4.2	2.5	3.8
Taenia saginata	0.3	0.0	0.0	1.3
Positive for one or more helminths	97.7	95.2	96.6	98.7

* One stool sample was collected from each individual. If both Lugol stained and fresh saline suspensions were negative, the zinc sulfate flotation technique was employed.

centrations of any one parasite from developing. Whatever the reasons may have been, specific correlations of parasites with hematological and blood vitamin values could not be established. Even in the case of hookworm infestation, the slightly lower hemoglobin (12.8 grams) and red blood cell (4.28 million) concentrations were not significantly different from those of persons without hookworm (13.3 grams., 4.38 million), nor did hematological values correlate with economic group, leadership status or type of farm. This was probably due to the multiplicity of factors present in the same individual which could influence hematological values. One-fourth of the entire group had hemoglobin values of 11.9 or below. Of these, 5 per cent were microcytic anemias (M.C.V. < 80) and 58 per cent were macrocytic (M.C.V. > 94).

Medical and dental findings. Of the total group of 945 examined on the basis of medical history alone, 21 per cent were considered to be in only fair health and 2 per cent in poor or very poor health. A similar impression was obtained from the physical examinations. The nutritional status was considered to be only fair in 21 per cent and poor or very poor in 4 per cent on gross inspection. However, over one-third of the entire group had exceedingly bad dentition, i.e., more than half of their teeth missing or carious, and only 22 per cent were without caries. Most of the latter were infants and young children. Gingivitis was observed in over one-fourth of the people but was considered due to poor dental hygiene rather than specific nutritional deficiency.

Although the riboflavin content of the diets was low, the serum riboflavin levels were within normal limits and the incidence of angular stomatitis (11 per cent) and seborrhea (2 per cent) was low. Mild to moderate circumlimbal vascularization of the cornea was observed in over one-fourth of the individuals examined, but the significance of this finding is uncertain. Approximately 40 per cent of the persons showed definite thickening and increased vascularization of the bulbar conjunctiva, but in Central American rural areas these signs are greatly influenced by chronic exposure to smoke in the houses and to dust during the dry periods. The frequent deficiency of vitamin A in the diet and the relatively low serum levels of carotene and vitamin A encountered suggest that inadequate intake of vitamin A may be a predisposing factor. The dry cracked skin of the extremities in nearly a quarter of the individuals and frank follicular hyperkeratosis in 12 per cent are at least consistent with this suggestion, although by no means diagnostic.

Five per cent of the population had palpable livers. Serum alkaline phosphatase values were not elevated in either adults or children. The absence of elevations in children agrees with the almost complete absence of skeletal deformities suggestive of rickets.

Although it is reasonable to assume that economic status, leadership and type of community influence both the serum vitamin levels and the many physical signs which were tabulated, the multiplicity of factors influencing these findings and the general lack of specificity of most of the physical signs are such that consistent and significant correlations could not be demonstrated, at least without detailed age and sex breakdowns beyond the scope of this introductory report.

Maternal and child health. The women in the study reported a total of 1,171 pregnancies during their lives. They stated that 11 per cent of these ended in abortions and 3 per cent in infants dead at or immediately after birth. In seventeen per cent of this total the children died during the first year after birth and in an additional 7 per cent they died before 14 years of age. Thus according to this information 449 of the products of the 1,171 pregnancies during the period represented by their reproductive life span were lost before adolescence, data unavailable to health authorities because many of these deaths would not be reported to them.

The reasons for such a high loss are many. Only 4 per cent of the births were attended by a physician and 20 per cent by an obstetrical nurse. The majority

were attended by untrained midwives. The inadequate diets of the mothers probably also increased the incidence of abortions and deaths from prematurity. Moreover, as soon as a child is born it is exposed to the dangers of a wide variety of intestinal infections[3] as well as parasites. All of the intestinal parasites discussed above have been found in preschool children.

It is also apparent that the nutrition of the infants is a precarious matter because of the uncertainty of the supply of mother's milk and the nature of the supplementary or substitute feeding provided. Thirteen per cent of the women were unable to nurse their babies and another 24 per cent had stopped nursing by the time the child was six months of age. At the age of one year 74 per cent of the babies were no longer receiving any breast milk; yet at this age only 64 per cent of them were receiving fruit juice, 46 per cent whole milk, 36 per cent cereal, 21 per cent egg and 9 per cent meat. Sugar water was given in significant quantities as a food to nearly half of these children. The educational problems relating to maternal and child care in such a situation are obvious.

Other factors listed as contributing to the mortality were whooping-cough, tetanus and diphtheria, all controllable by proper inoculations, and pneumonia, measles and malaria which ordinarily should not be fatal with adequate treatment. A further indication of the lack of medical attention is the fact that no cause of death could be suggested by the mother for approximately half of the deaths occurring between birth and 14 years of age.

The community approach to solutions. Turrialba has developed through the years an increasingly complicated pattern of health systems designed to meet these and other fundamental health problems. This pattern combines characteristics of the simpler community systems, such as the "curandero" (folk practitioner) and "partera" (mid-wife), with the relatively elaborate Social Security Hospital and the Public Health Unit. In addition, a physician and dentist have private offices in the town. In all, five medical doctors serve a population of nearly 30,000 inhabitants and the pharmacists are called upon frequently for advice on medical problems.

Inquiry as to the number of visits made by persons in the study for medical purposes during 1948 was included in the health questionnaire and tabulated by percentage of visits to each health service. Differences were observed in the habits of people of "hacienda" and small and medium farm neighborhoods (Table 4). This was due primarily to the employer-employee sharing of the Social Security quotas. Small farmers operate in both capacities and many seem to prefer to run their own health risks. In general, the data seem to indicate that the rural population prefers professional services when available, but is willing to use non-professional advice when other cannot be secured.

[3] In a recent survey carried out by the Ministry of Health in cooperation with the Tropical Research Foundation of Chicago, 46 individuals in the Turrialba study furnished a single fecal sample for culture. In this small group, *Proteus*, *Shigella sonnei*, *Salmonella wichita*, *S. typhisius*, *S. muenchen*, and *Paracolon* (Arizona and Bethesda types) were identified. Of 19 employees of the Inter-American Institute of Agricultural Sciences in Turrialba, examined at the same time, one was found to have *Sh. alkalescens* and another *Sh. paradysenteriae* Flexner IIB (Wb).

In simpler communities, health systems arise from the direct efforts to meet the needs of the people as they see them. These systems are also developed in close harmony with the economic means and other fundamental characteristics of the culture. In more evolved communities, this harmony with "felt needs", economic means and other cultural characteristics is not as close because *responsibility for program formulation and execution is largely removed from the community to the state or national levels.* The result usually takes the form of numerous programs bearing on health, uncoordinated at the community level, failing to meet adequately the "felt needs" of the people and sometimes confusing them. On the other hand, the local population loses the ability to comprehend the technological and economic limits placed on community services

TABLE 4

Use of different health systems in two types of neighborhoods, Rural Zone, Turrialba, Costa Rica, 1948

SYSTEMS	PER CENT OF VISITS	
	Large hacienda type neighborhood	Neighborhood of small and medium farms
Social Security Hospital	62	47
Public Health Unit*	2	10
Private dentist	3	5
Private physician	3	8
Obstetric nurse	0	1
Mid-wife	2	3
Folk practitioner	9	7
Pharmacist	19	19
Total	100	100

* At the time the questions were asked, the Public Health Unit in Turrialba was not active. It has since been reorganized and well staffed, partly as a result of the needs revealed by this study.

by the resources and degree of development of the country. Their demands for services often surpass even the most visionary estimates of potentialities.

Turrialba is an example of the operation of these factors. Most of the heads of families interviewed in 1948 reported that there were fundamental deficiencies in the services offered by the two most important community health agencies, the Social Security Hospital and the Health Unit.[4] The people expected curative services from both agencies, even though the Health Unit's program stressed preventive medicine.[5] They did not seem to appreciate the financial and technical limitations under which both agencies must work.

The health problems reported above, on the other hand, are ample evidence

[4] See footnote, Table 4.

[5] Also observed by George Foster in a 1951 report of the Institute of Social Anthropology in Washington, D. C., "A Cross-cultural Anthropological Analysis of a Technical Aid Program", p. 65-66.

that the health needs of the families were not being adequately met by existing services, although many governmental departments and agencies were active in the area. The maximum use of the limited resources available is not obtained because the activities of the school system, the agricultural organizations, and the health agencies are not fully integrated at the community level. The closest possible cooperation of all these agencies is required for a more effective approach to the health problems revealed by the study. It is evident that better nutrition, control of parasites, improvement of water supplies and sanitary facilities, and improved maternal and child care are all necessary in the Turrialba community.

The specific public health measures developed to meet the situation must be integrated in the broader community development program. In Turrialba an attempt is now being made to do this by placing emphasis on the following operational principles.

1. *Increasing the participation of local people, both rural and urban, in program formulation and execution.*

2. *Promoting closer cooperation among agencies serving the community.*

3. *Reaching the entire population with simple and economical methods of health education and service.*

As the Turrialba Community Development Study progresses, experience will be accummulated in the practical application of these principles to action programs. Their application is believed essential for the permanent success of all efforts to improve public health on a community or area basis, particularly in those areas which are undergoing an active transition to more evolved health systems under the stimulus of international programs.

SUMMARY

The Turrialba, Costa Rica, Community Development Project was initiated in 1947 with studies of economic and social structure. 140 families were selected from the rural population for health and dietary studies during 1948–51. This sample included the major economic, leadership and geographical groups on a proportionate basis and was composed of 940 individuals. This group was also used in 1951 for detailed clinical, dental, fluoroscopic, parasitological and blood vitamin and protein studies. The major public health problems included dietary deficiency of vitamin A, riboflavin, calcium and animal protein; very high incidence of multiple intestinal parasitism; unsatisfactory drinking water, sanitary and bathing facilities; poor infant feeding practices and general ignorance of the basic principles of hygiene and nutrition.

The Turrialba Rural Community development study is being continued, and efforts are being made to improve the integration of all agencies concerned with the project; to secure increased participation of local people in program formulation; and to adapt or develop simple and economical methods of health education and service to reach the entire population. These measures are considered essential for the lasting success of national or international efforts to improve public health on a community or regional basis.

AN ANALYSIS OF A MEMORANDUM ON THE DIAGNOSIS OF AMEBIASIS

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INTRODUCTION

Several years ago through the efforts of Dr. Joseph D'Antoni, a committee on amebiasis was formed to consider various phases of the disease. Although it was composed of members of the American Society of Tropical Medicine and received the encouragement and support of the Society, it did not have official status in the Society. In 1950 the authors of this paper were requested to devote their attention to the problems of amebiasis.

The clinical and laboratory diagnosis of amebiasis is difficult and from the rather extensive literature on the subject there would appear to be little or no agreement on the methods that should be used and on the significance of various observations. This lack of accord was evident at the Panel Discussion on Amebiasis held during the annual meeting of the American Society of Tropical Medicine, December 7, 1948 (Amer. Jour. Trop. Med. **30:** 121–169. 1950). In view of the confusion that exists, the authors considered that it was important to obtain and analyze current opinions expressed by those active in the field of amebiasis in the United States. The accompanying analysis of a memorandum on the diagnosis of amebiasis does not constitute an official document of the Society, but an independent publication of the authors.

PROCEDURES

Memorandum on the diagnosis of amebiasis. A memorandum on the diagnosis of amebiasis, consisting of 115 statements or questions, was prepared (see Appendix). Although it was constructed in the style of a questionnaire, the purpose was not simply to obtain votes *pro* and *con* but to obtain definite opinions which could be analyzed and interpreted. Following 106 of the statements there was space to indicate agreement, disagreement, or no opinion, relative to the statement, and to cite whether the answer was based upon "impressions gleaned from the literature" or upon "comparative data obtained through experience." There also was space for the respondent to present evidence in support of his contentions. He was encouraged to present actual data or information from his experience. The statements as presented in the memorandum did not necessarily represent the opinions of the authors and in some instances the statements were intentionally provocative. The memorandum included 9 questions which required narrative responses.

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Although the memorandum was divided into clinical diagnosis and laboratory diagnosis, a majority of the statements were concerned with the latter. The laboratory diagnosis was divided into two sections, one devoted to the indirect method, utilizing the complement-fixation test, and the other the detection of *Endamoeba histolytica* through microscopy, including methods of collection, preservation and examination.

Distribution of the memorandum. The first draft of the memorandum was distributed to the members of the "Amebiasis Committee" and to a number of individuals suggested by its members. The preliminary analysis of the 45 replies received was presented at the business meeting of the American Society of Tropical Medicine at its annual meeting in 1950. The Council recommended that the memorandum be distributed to all of the members of the Society and provided funds for this purpose. Unexpected foreign assignments of certain of the authors prevented the distribution until the fall of 1951.

In distributing the memorandum to all of the members of the Society, it was not the intention to obtain a poll of all the membership. The purpose was to reach a greater number of individuals with specialized interest in amebiasis. The sheet of explanation that accompanied the memorandum encouraged only those members with "interests and experience" in the field of amebiasis to complete and return the memorandum.

Realizing that a person might feel qualified on one aspect of the subject and not on another, the explanation sheet contained the following directions: "Please do not feel that it is necessary to give a reply to each statement. Although we are anxious to obtain as much information as possible, we realize that the memorandum is lengthy and that very few individuals, due to their specialized interests, will be in a position to give an opinion on each statement; therefore, you need to reply only to those statements on which you feel you have adequate background."

RESULTS

A total of 93 memoranda were completed by members of the "Amebiasis Committee" (plus selected individuals) and members of the American Society of Tropical Medicine.[7] There were no significant differences of opinions of the two groups and therefore the two groups will be considered as a single unit. A separate analysis was made of the replies of those individuals who indicated that their answers were based upon experience. However, in order to simplify this report, the results of this analysis will not be presented except in the few instances where it was significantly different. The percentage of approval of each statement is based upon the number of definite replies to the specific question. Thus, only the responses of "agree" or "disagree" were counted. There was not a single statement to which all 93 individuals responded. Presumably they attempted to adhere to the suggestion that they restrict their replies to those statements only on which they considered themselves qualified. The greatest number of replies (87) was received on statement 105 and the smallest number (16) on statement 75.

[7] A list of the names of the respondents is available from the senior author upon request.

The percentage approval on the various statements ranged between 6 and 100 per cent. If it is assumed that the positive or negative wording of the statement did not influence the answers significantly, 6 per cent approval represents 94 per cent disapproval of the statement which can conveniently be expressed as 94 per cent agreement to the negative of the statement. In other words, the further away from 50 per cent approval toward either 100 or 0, the greater the agreement by the group responding. If 50 per cent approved and 50 per cent disapproved there was no agreement by the group relative to the statement.

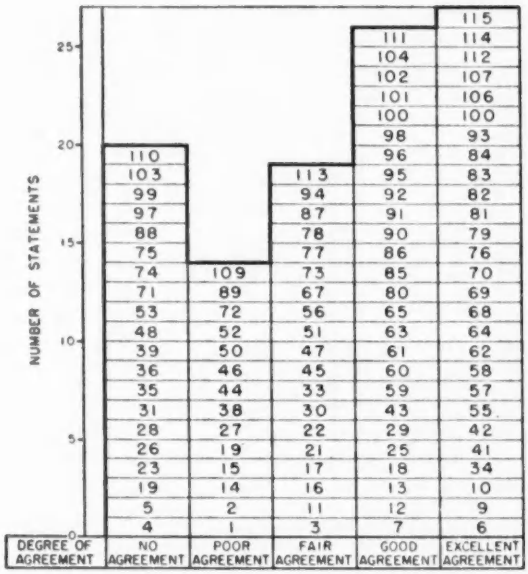

FIG. 1. Number of statements with various degrees of agreement based upon the percentage of respondents approving of statement. Numerals within columns represent the number of the statement in the memorandum (Appendix).

For the sake of analysis, the scale of approval from 51 to 100, and 50 to 0, has been divided as follows to represent various degrees of agreement:

51 to 60 and 41 to 50 represents	no agreement
61 to 70 and 31 to 40 represents	poor agreement
71 to 80 and 21 to 30 represents	fair agreement
81 to 90 and 11 to 20 represents	good agreement
91 to 100 and 1 to 10 represents	excellent agreement.

In Figure 1, the 106 statements which required an "agree" or "disagree" reply are divided according to the degree of agreement expressed by the individuals responding. Exactly 50 per cent of the statements are within the "no", "poor", and "fair" columns, and 50 per cent are within the "good" and "excellent" columns. The percentage agreement for those *citing experience* produced a

graph essentially the same as that in Figure 1 which considered all replies. On an average, the percentage approval was approximately one-half of 1 per cent higher for the "experience" replies, and with all except 21 statements, the degree of agreement was the same for both groups. In only one instance did the degree of agreement change more than one grade.[8]

The statements in the memorandum can be divided into 13 categories according to the subject matter discussed. Table 1 presents the number of statements in each category that had different degrees of agreement. The best agreement

TABLE 1

Number of statements in various categories with different degrees of agreement

Categories of Statements	No. of Statements per category	Number of Statements with designated Degree of Agreement				
		No	Poor	Fair	Good	Excellent
1. Clinical Diagnosis	8	2	2	1	1	2
2. Complement Fixation	3			1	1	1
3. Ident. in Feces - General	5		2	2	1	
4. Ident. in Feces - Trophozoites	13	5	2	3	3	
5. Ident. in Feces - Cysts	6	3	1	1		1
6. Ident. in Culture - General	5		1	1	1	2
7. Ident. in Culture - Trophs & Cysts	7	2	3	2		
8. Collection of Specimens	11			1	5	5
9. Storage and Preservation	5	1		1		3
10. Exam. Techniques - Use of Procedures	23	3	2	5	6	7
11. Exam. Techniques - Selection of "Routine" or "Supplementary"	7	2			5	
12. Exam. of Multiple Specimens	6	1			2	3
13. Laboratory Reports	7	1	1	1	1	3
TOTAL NUMBER OF STATEMENTS	106	20	14	19	26	27

was obtained on those questions concerned with the collection of specimens. "Good" or "excellent" agreement was obtained on 10 of 11 statements in this category. On 5 statements there was "excellent" agreement. A rather even distribution of the statements between "no" agreement and "excellent" agreement occurred in those categories concerned with clinical diagnosis and the application of the various examination technics (categories 1 and 10). The poorest agreement was obtained on those categories concerned with the identification of tro-

[8] For statement 3 the "experience" replies were in "excellent" agreement (91%) while all replies were in only "fair" agreement (77%): "In the absence of positive laboratory evidence, the use of therapeutic tests may assist diagnosis under certain conditions."

phozoites and cysts in feces and culture (categories 4, 5, 7). Of 26 statements in these categories, on only 4 was there "good" or "excellent" agreement.

In replying to the statements on the identification of *E. histolytica*, if a person disagreed that the characteristics constituted conclusive proof, he might have indicated that the characteristics constituted presumptive evidence in need of further confirmation,[9] or even less than presumptive evidence. In disagreeing with a statement of presumptive evidence, a person might have maintained that he considered the statements less than presumptive or conclusive. An analysis of the replies to these statements is presented in Tables 2 and 3. It should be noted in both tables that by far the greatest number of individuals disagreeing with the statements felt that the characteristics were less than presumptive rather than conclusive or presumptive. There were very few instances when the characteristics of presumptive evidence were considered to be conclusive (Table 3). In only two instances did a greater number of the individuals feel that the conclusive proof cited was presumptive rather than less than presumptive (statements 21 and 47 in Table 2). According to the number who considered the conclusive proof cited in statements 21, 33 and 47 as presumptive, apparently the responding individuals would have demonstrated good agreement on these statements had they been indicated as constituting presumptive evidence rather than conclusive.

It will be noted that there was "excellent" agreement (95 per cent) with only one set of criteria (statement 34, Table 2) listed as conclusive proof of *E. histolytica*. This statement in full is: "Cysts larger than 10 microns containing 1 to 4 *Endamoeba*-type nuclei and rod-shaped chromatoid bodies with smooth rounded ends (combination saline and iodine or permanent stained smears)." "Good" agreement (84 per cent) was the best recorded for any of the listed criteria as conclusive proof for the trophozoite stage of *E. histolytica* (statement 18, Table 2). This statement in full is: "Trophozoites that contain red blood cells, extrude hyaline pseudopodia, and exhibit progressive locomotion (saline smears)." There was no statement concerning the identification of *E. histolytica* in culture with which there was either "excellent" or "good" agreement. Although trophozoites predominate in culture, it is interesting to note that the best agreement on the identification in culture dealt with the cysts. This statement (47) which had only fair agreement (79 per cent) is similar though not identical with statement 34 above. Statement 47 in full is: "Cysts with rod-shaped chromatoid bodies with smooth rounded ends (saline or permanent stained smears)."

In Table 4 is presented an analysis of the replies of the individuals who disagreed with the "routine" or "supplementary" use of the technics as indicated by the memorandum. There was "good" agreement as to the use of all of the procedures with the exception of the iodine smear and concentration technics (statements 97 and 99). There was "no" agreement that these two technics constituted supplementary technics since only about half of the individuals (41 and 44 per cent respectively) responding agreed with the statements. In

[9] "Presumptive evidence in need of further confirmation" was interpreted in the memorandum as "probably *E. histolytica* but not absolutely certain."

TABLE 2

Statements of characteristics cited in memorandum as conclusive proof of E. histolytica *showing number of those disagreeing who considered the characteristics either presumptive (evidence) or less than presumptive*

Statement No.	Conclusive Proof of E. histolytica (Abbreviated Statements)	No. of Replies	Degree Agreement *	No. Disagreeing	Considered	
					Presumptive	Less than Presumptive
	Trophozoites in Fecal Specimens					
18.	Red blood cells; hyaline pseudopodia; progressive locomotion.	79	Good(p)	13	1	6
19.	Inactive; containing red blood cells.	69	No	38	3	11
20.	In old specimen - progressive locomotion, no rbc's.	61	Poor(n)	39	6	12
21.	No cytoplasmic inclusions, classical histolytica nucleus.	75	Fair(p)	16	5	1
22.	Red blood cells, no bacteria, Endamoeba-type nucleus.	73	Fair(p)	19	3	3
23.	Less than 10 microns, Endamoeba-type nucleus.	59	No	25	8	10
	Cysts in Fecal Specimens					
33.	Less than 10 microns, rod-shaped chromatoid bodies.	78	Fair(p)	20	5	6
34.	Larger than 10 microns, 1 to 4 Endamoeba-type nuclei, rod-shaped chromatoids.	79	Exc.(p)	4	1	2
35.	Four Endamoeba-type nuclei, no chromatoid bodies.	75	No	35	13	16
36.	Regardless of size and number of nuclei, rod-shaped chromatoid bodies.	70	No	37	8	15
	Organisms in Culture					
46.	Trophozoites - progressive locomotion, Endamoeba-type nucleus.	57	Poor(p)	22	4	14
47.	Cysts - rod-shaped chromatoid bodies.	79	Fair(p)	12	5	4
48.	Cysts - four Endamoeba-type nuclei.	59	No	23	6	16

* Positive agreement, under "Degree of Agreement," is indicated by "(P)" and negative agreement by "(N)".

both instances the greatest majority of those disagreeing (31 of 45 and 30 of 43) felt that these technics should be used routinely. However, it is interesting to note that in both instances 13 (over 28 per cent) of those disagreeing felt that the technics had little or no value.

TABLE 3

Statements of characteristics cited in memorandum as presumptive evidence of E. histolytica *showing number of those disagreeing who considered the characteristics as either conclusive proof or less than presumptive evidence*

Statement No.	Presumptive Evidence of E. histolytica (Abbreviated Statements)	No. of Replies	Degree Agreement*	No. Disagreeing	Considered	
					Conclusive	Less than Presumptive
	Trophozoites in Fecal Specimens					
25.	In warm specimen - progressive locomotion.	73	Good(p)	13	1	7
26.	Invisible nucleus; no apparent cytoplasmic inclusion.	73	No	31		22
27.	"Slug" shape; no visible locomotion.	59	Poor(n)	36		32
28.	Clear, finger-shaped pseudopodia; no progressive locomotion.	71	No	37		26
29.	Histolytica-type nucleus; no bacteria.	69	Good(p)	10		7
30.	Coli-type nucleus; no cytoplasmic inclusions.	65	Fair(n)	48		27
31.	Coli-type nucleus; red blood cells.	64	No	28	1	19
	Cysts in Fecal Specimens					
38.	Cysts each having a single Endamoeba-type nucleus.	72	Poor(p)	23	1	17
39.	Larger than 10 microns; neither nuclei nor chromatoids visible.	72	No	35		25
	Organisms in Culture					
50.	Easily cultivated.	52	Poor(p)	20		14
51.	Sluggish trophozoites, Endamoeba-type nucleus (invisible in saline).	53	Fair(p)	15	1	6
52.	Cysts having single Endamoeba-type nuclei.	52	Poor(p)	18	2	8
53.	Cysts larger than 10 microns; invisible nuclei.	48	No	20	1	9

* Positive agreement, under "Degree of Agreement," is indicated by "(P)" and negative agreement by "(N)".

Sixty-eight individuals replied to statement 108, "In your experience what is the optimum number of specimens to assure maximum efficiency of diagnosis?" One person indicated that one specimen was sufficient, thirty-five indicated three or four and thirty-two preferred five or more. Sixteen individuals stated

that one or more of the specimens should be collected after catharsis and five maintained that sigmoidoscopy should be used. In reference to the other statements requiring a written reply, not enough individuals responded to justify an analysis.

TABLE 4

Statements of the utilization of diagnostic procedures showing number of those disagreeing with the indicated use who considered that the procedure should be used routinely, as a supplementary technique, or that the procedure has little or no value

Statement No.	Selection of "Routine" and "Supplementary" Procedures (Abbreviated Statements)	No. of Replies	Degree Agreement *	No. Disagreeing	Considered		
					Routine	Supplementary	Little or no value
95.	Saline smear.."routine" procedure.	77	Good(p)	10	/	5	3
96.	Aqueous smear.."supplementary" procedure.	60	Good(p)	11	0	/	3
97.	Iodine smear.."supplementary" procedure.	76	No	45	31	/	13
98.	Other wet stained smears.. "supplementary" procedures.	67	Good(p)	8	4	/	0
99.	Concentration techniques.. "supplementary" procedures.	77	No	43	30	/	13
100.	Cultivation.."supplementary" procedure.	75	Good(p)	9	3	/	2
101.	Permanent stained smear.. "supplementary" procedure.	79	Good(p)	13	9	/	3

* Positive agreement, under "Degree of Agreement," is indicated by "(P)" and negative agreement by "(N)".

DISCUSSION

Although not entirely unexpected, it is nevertheless a little surprising and of no little concern that there was "good" and "excellent" agreement on only one-half of the statements in this memorandum dealing with the clinical and laboratory diagnosis of amebiasis. The lack of agreement that is apparent from the replies probably indicates that we still do not have reliable basic information on many aspects of the subject. It is hoped that one of the benefits that may arise from this memorandum will be to focus attention upon some of the problems that exist. The recognition of present-day inadequacies should stimulate research to clarify specific points.

The authors recognize that agreement by a group of individuals does not in itself constitute truth. It may very well be that a number of the statements with "good" or "excellent" agreement should be studied again, but practically all of those with only "fair" agreement or less deal with aspects of the subject which

need careful investigation. On approximately 20 per cent of the statements there was evidence of marked controversy in which just about as many persons disagreed as agreed. Not all of the problems can be discussed in this analysis; however by noting the extent of agreement (or rather lack of agreement) reached by the individuals responding (Appendix and Figure 1), many of the current problems will be apparent to the reader.

In the following discussion, the degrees of agreement of "good" and "excellent" have been considered as representing the beliefs of the individuals responding to the statements. Although the general beliefs will be stressed in this section, a number of points of dissension will be presented. For the sake of emphasis, the identification of the organism will be presented before the other categories of statements.

Identification of E. histolytica *in feces* (*statements 13–40*). One of the most obvious difficulties concerns the characteristics which are adequate for differentiating *E. histolytica* from the other amebae. From the lack of agreement relative to this subject (statements 13–54), one wonders by what means the laboratory diagnosis has been accomplished during the past 50 years or more. In view of the confusion it would appear advisable that each characteristic or group of characteristics should be carefully studied by competent investigators in order to determine their relative value and reliability. The recent work by Hoare (Parasitology **42:** 43–47, 1952) on the cytoplasmic inclusions of *E. histolytica* trophozoites is an excellent example of what should be done with the other characteristics.

It appears from the responses to the several statements that more reliance is placed on the characteristics of the cyst for the identification of the species in feces. There was excellent agreement (95 per cent) that statement 34, "Cysts larger than 10 microns containing one to four *Endamoeba*-type nuclei and rod-shaped chromatoid bodies with smooth, rounded ends (combination saline and iodine, or permanent stained smears)" constituted conclusive proof of *E. histolytica*. However, a similar statement (33) which did not specify anything about the nuclei but did specify forms less than 10 microns had only fair approval (74 per cent). Was size the important issue, or number of nuclei, or both? A few people stressed that *histolytica*-type nuclei was an essential point and that *Endamoeba*-type nuclei was not sufficient. Is it possible that there is universal agreement on a set of cyst characters which was approached but not reached in statement 34 above? Probably the classical description would have satisfied everyone; however, since all of the classical features are seldom observed, the memorandum attempted to evaluate certain combinations of characteristics which are more frequently seen in the usual types of preparations.

While there was good agreement (84 per cent) that a combination of hyaline pseudopodia, progressive movement and ingestion of red cells (statement 18) constituted conclusive proof of *E. histolytica*, a few people stressed the additional need of seeing a specific *histolytica*-type nucleus in a stained specimen. These apparently would not accept any diagnosis of unstained trophozoites in fresh fecal preparation as more than presumptive.

Identification of E. histolytica in cultures (*statements 41–54*). The responding

individuals agreed that it is necessary to differentiate organisms in a culture by morphological characteristics since there are no strictly selective media for *E. histolytica* and no reliable food particles ingested by the organisms. To add to the problem, they also agreed that in culture *E. histolytica* may be motionless and *Endamoeba coli* may exhibit progressive locomotion. There was no statement relative to the identification in culture that they believed constituted conclusive proof of *E. histolytica*. Apparently they agreed that "cysts with rod-shaped chromatoid bodies" (statement 47) was at least presumptive evidence of this species. Presumably if the statement had included "1 to 4 *Endamoeba*-type nuclei" as did statement 34 they would have considered the characteristics as excellent for conclusive proof. It is interesting that although trophozoites predominate in culture and are the usual stage used for identification by this technic, "poor" agreement was obtained on the following statement 46, "Majority of trophozoites exhibiting progressive locomotion (saline smears) and with *Endamoeba*-type nuclei (temporary wet or permanent stained smears)."

Although there appeared to be very little agreement as to the value of the cited characteristics of *E. histolytica* in feces and culture, there were only a few attempts on the part of the respondents to formulate more precise sets of criteria for the identification of the species in replies to questions 24, 32, 37, 40, 49, and 54, which were designed for this purpose.

Clinical diagnosis (*statements 1–9*). The respondents did not believe that any drug other than emetine has been sufficiently evaluated as a therapeutic test. As a matter of fact, there was no agreement on the value of emetine in such a test (statement 4). Only 77 per cent of all of the respondents agreed that the "therapeutic tests" may be of assistance in the diagnosis of amebiasis (statement 3). However, of the limited number (32) who cited experience with this procedure,91 per cent (excellent) agreed that it was of some value.

As a group, the responding individuals did not believe (87 per cent) that any combination of nonspecific symptoms such as "chronic fatigue, right lower quadrant discomfort, abdominal distention, colic, nausea and loss of weight are pathognomonic of amebiasis" or that their disappearance following specific therapy constitutes conclusive proof that *E. histolytica* was responsible.

Complement-fixation test (*statements 10–12*). Although the individuals replying to the statements could not agree on the value of the CF test in the diagnosis of intestinal amebiasis, they agreed that it is a useful adjuvant in the diagnosis of extra-intestinal involvement. However, they did not believe that a strong positive necessarily constitutes a diagnosis of amebiasis.

Collection of specimens (*statements 55–66*). There was "good" or better agreement on all but one of the statements relative to the collection of specimens. Since only a few pertinent points will be discussed, other details can be obtained by referring to the Appendix and Figure 1. The group agreed that the normally passed stool is a desirable specimen for an initial examination. Although there was some reservation on the value of post-cathartic specimens, they agreed that when catharsis is used, at least three immediate post-cathartic specimens should be collected and examined for *cysts*, as well as for trophozoites. They did not

believe that sigmoidoscopy should be employed until other means had failed to reveal *E. histolytica*.

Storage and chemical preservation of specimens (*statements 67–71*). The respondents believed that if storage for a few hours is necessary, the specimens should be maintained at room temperature, and for longer periods should be placed in the refrigerator, but that lower temperatures have not proved satisfactory. They agreed that if a delay of four hours or more is necessary, a portion of the specimen should be mixed with PVA-fixative to preserve the trophozoites. There were apparently conflicting opinions relative to the need of preserving cysts during transportation of stool specimens by mail.

Examination technics (*statements 72–94*). The individuals responding agreed that the iodine smear is helpful in the identification of cysts and that wet stained smears especially designed for amebic trophozoites may be helpful in distinguishing *Endamoeba* from other genera. However, they did not believe that the wet stained smears could be relied upon to differentiate trophozoites of *E. histolytica* and *E. coli*.

There was "excellent" agreement on all four statements relative to the application of concentration procedures (see Appendix). Of particular interest, they believed that if no organisms are found in the wet smears, a concentration should be run in order to recover rare cysts that may be present.

Although the group agreed that the older the specimen the more difficult is cultivation of the cysts that are present, they believed that the procedure may be helpful in diagnosing specimens containing "only degenerating trophozoites by making possible the production of more normal progeny" (statement 86). They knew of no reliable routine procedure for provoking encystation of amebae in culture.

The respondents believed there is little likelihood of finding *E. histolytica* organisms in permanently stained smears if they cannot be demonstrated in wet smears and by concentration technics. They agreed that permanent preparations are helpful in revealing the details of the nuclei and the shape of the chromatoid bodies.

Selection of "routine" and "supplementary" procedures (*statements 95–101*). The persons replying to the statements maintained that the saline smear should be used routinely on all specimens and that the aqueous smear, wet stained smears (other than iodine), cultivation, and permanently stained smears should be employed as supplementary procedures, as needed. There was some question concerning the utilization of the iodine smear and concentration procedures. There was no agreement on their being supplementary procedures and a large proportion of those disagreeing felt that they should be used routinely (Table 4).

Examination of multiple specimens (*statement 102–108*). The group recognized the limitations of single specimens regardless of the method of collection and agreed to the importance of examining repeat specimens. They felt, in general, that the number of repeated specimens should be governed by the clinical suspicion of amebiasis. All but one of the 69 individuals responding stated that three or more specimens should be examined to assure maximum efficiency.

Laboratory reports (*statements 109–115*). The responding individuals believed that all species of organisms found should be reported to the physician and that the report should include information on the type and condition of the specimens received by the laboratory. They agreed that blood, mucus, pus cells, macrophages, Charcot-Leyden crystals, should be reported and that instead of "negative" a statement such as "no parasites found" or "N.P.F." should be entered if the examination failed to reveal any organisms.

Summary of problems. Although a number of the major problems in the diagnosis of amebiasis have been presented above, there was lack of agreement on many other points. Several of these are included below in a list of questions. While a number of the problems illustrated by the questions have been carefully investigated already by various workers, from the replies to this memorandum it is evident either that the published results are not widely known or that they have not been generally accepted.

"Are there any physical signs, symptoms or clinical tests which can justify clinical diagnoses of amebiasis?"

"What is the value of the complement-fixation test in intestinal amebiasis?"

"Are there consistently reliable characteristics that can be used to identify the trophozoites and cysts of *E. histolytica*?"

"Can selective media be developed to support the growth of only *E. histolytica*?"

"Is there a method of routinely causing amebae to encyst in culture?"

"Are there any circumstances, and if so what, under which specimens should be kept artificially warm before they are examined?"

"Is there a need for the preservation of cysts during shipment of specimens by mail?"

"Of what value are the aqueous and saline wet mounts in the examination of fresh specimens?"

"Should the iodine wet mount and concentration technique be used routinely on all specimens?"

"To what extent are chromatoid bodies visible in iodine mounts?"

"Can iodine mounts be used in the examination of amebic trophozoites?"

"What staining procedure is best for permanent mounts? Should this technic be used routinely?"

"Can the oil immersion objective be used with advantage on wet mounts?"

"Will the overnight storage of feces containing cysts in tap water reveal otherwise invisible chromatoid bodies?"

"Under what circumstances should cultivation be employed to assist in *finding* the amebae when they are not otherwise evident?"

"How effective is purgation in obtaining evidence of infection?"

"Should distinction be made in laboratory reports between large and small races of *E. histolytica*?"

COMMENTS

Although the results of this memorandum would indicate that our present knowledge of the diagnosis of amebiasis is in a confused state, the authors believe that this is probably more apparent than real. The replies demonstrate again that it is difficult to get black or white commitments from scientists. Awareness of the rare exceptions weigh heavily on the mind when asked either to agree or disagree with a statement and consequently one wishes to qualify his responses. This was evident in the comments of some of the respondents. It is realized that a "yes" or "no" reply on a number of the statements was practically impossible. Since "yes" indicated complete agreement, "no" in many instances probably meant "not complete agreement;" it perhaps only occasionally meant "complete disagreement." Some of the respondents may not have replied to statements because of this dilemma. Although the authors have attempted whenever possible in the discussion to take into account the qualifying comments of the respondents, it is recognized that the degree of agreement to the statements in the various tables may be misleading because of the inability to express shades of opinion in "yes" or "no" replies. The authors feel that reliable diagnoses of amebiasis are being made in many places within the United States. However, until we can establish common reproducible criteria which do not need to be qualified, the diagnosis will appear to be based more upon subjective rather than objective evidence.

SUMMARY

A memorandum consisting of 115 statements on the clinical and laboratory diagnosis of amebiasis was prepared and distributed to the members of the American Society of Tropical Medicine. The purpose of the memorandum was to obtain for analysis the current opinions on the subject expressed by those active in the field of amebiasis in the United States. Only those members with "experience and interest" in the subject were encouraged to complete and return the memorandum. The current report presents an analysis of replies submitted by 93 individuals.

Those responding demonstrated "good" or "excellent" agreement on only one-half of the statements. The poorest agreement was indicated on those statements concerned with the identification of trophozoites and cysts of *E. histolytica* in feces and cultures. It is apparent from the replies that each characteristic or group of characteristics currently used in identification should be carefully studied by competent investigators in order to determine their true value and reliability.

In addition to citing other problems which need careful investigation, the report summarizes the general beliefs of the respondents as evident from the replies to the various statements in the memorandum.

ACKNOWLEDGEMENT

The authors wish to express their appreciation for the interest and advice which was generously given by the late Dr. E. G. Hakansson during the preliminary preparation of the memorandum.

APPENDIX

List of 115 statements distributed to members of the American Society of Tropical Medicine in 1951 (The number of each statement is shown at the right in bold face figures)*

I. *Clinical Diagnosis*—The final diagnosis of amebiasis depends upon the physician's judgment and his interpretation of clinical and laboratory evidence.

A. In view of the difficulty of obtaining accurate laboratory diagnosis and in order not to delay treatment of patients:

1. The clinical diagnosis of acute amebic dysentery is justified. **1**
2. The clinical diagnosis of amebic involvement of the liver is justified when supported by roentgenologic evidence. **2**

B. In the absence of positive laboratory evidence, the use of therapeutic tests may assist diagnosis under certain conditions. **3**

C. The abatement of acute symptoms of amebic dysentery and amebic involvement of the liver within 48 hours after one or two intra-muscular injections of emetine hydrochloride (1 mg. per kilo body weight daily; not to exceed 65 mg. per day) constitutes a reliable therapeutic test for these conditions and when other evidence is unavailable, the clinical diagnosis may be considered to be confirmed. **4**

D. Although the response is slower and less dramatic, the clinical improvement following the oral administration of chloroquine constitutes a reliable therapeutic test to confirm the diagnosis of amebic involvement of the liver. (If you agree, indicate desired dosage.) **5**

E. At the present time, no other drug has been sufficiently evaluated as a therapeutic test. **6**

F. Non-specific symptoms such as chronic fatigue, right lower quadrant discomfort, abdominal distention, colic, nausea and loss of weight are not pathognomonic of amebiasis. **7**

G. State any other symptom or set of symptoms which you would accept as pathognomonic of non-dysenteric amebiasis. **8**

H. Disappearance of non-specific symptoms following specific therapy for amebiasis is conclusive proof that *E. histolytica* was responsible for the clinical findings. **9**

II. *Laboratory Diagnosis*—With the possible exceptions of acute amebic dysentery and amebic involvement of the liver, clinical diagnosis of amebiasis should be confirmed by positive laboratory findings.

A. *Complement-Fixation Test*—As with all serological diagnostic procedures, the complement-fixation test for amebiasis is a presumptive test which should be supported, whenever possible, by the demonstration of *E. histolytica* and by clinical evidence.

1. The C-F test is a useful adjuvant in the diagnosis of extra-intestinal amebiasis. **10**
2. The value of the C-F test in the diagnosis of intestinal amebiasis has not been demonstrated. **11**
3. A strong positive C-F test does not necessarily constitute a diagnosis of amebiasis. **12**

B. *Demonstration of Endamoeba histolytica*—Within the limits of our present knowledge the Subcommittee will assume that the etiological agent of amebiasis is *E. histolytica*

* Replies were received from 93 persons with specialized interest in amebiasis, containing expressions of opinion on the statements. These opinions are analyzed in Fig. 1 and Tables 1–4.

and will confine its attention to the recovery and identification of that organism. Since the object of the laboratory procedures is to obtain organisms that can render a diagnosis possible, the identification of the organism will be considered first.

1. *Identification of Organism in Fecal Specimens*
   a. There is no single characteristic that is reliable for the identification of *E. histolytica* trophozoites or cysts. **13**
   b. Red blood cells occur so rarely within the trophozoites of *E. histolytica* observed in the United States that this characteristic can seldom be used in identification of the organism. **14**
   c. Nuclear morphology alone even in permanent stained smears is not adequate for identification of *E. histolytica* trophozoites or cysts. **15**
   d. The locomotion of amebic trophozoites is less typical in purge or enema specimens than in those spontaneously passed. **16**
   e. In general, for final identification of *E. histolytica*, the cystic stage is more reliable than the trophozoites. **17**
   f. *Trophozoite Stage of E. histolytica.* In replying, indicate the geographical location of your experience and the type of population examined.
      1) *Conclusive Proof*—If you disagree with any of the following statements, please qualify your answer by giving your evaluation of the characters cited.
         a) Trophozoites that contain red blood cells, extrude hyaline pseudopodia, and exhibit progressive locomotion (saline smears). **18**
         b) Inactive trophozoites containing red blood cells (saline smears). **19**
         c) Trophozoites in a relatively old specimen, (i.e. 4 to 16 hours) exhibiting definite rapid progressive locomotion, but containing no red blood cells (saline smears). **20**
         d) Trophozoites containing no cytoplasmic inclusions, but with nuclei containing a small, centrally-placed karyosome and small, evenly distributed peripheral chromatin granules as revealed in (1) stained wet mounts and in (2) permanent stained smears. **21**
         e) Trophozoites containing red blood cells, no (or few) bacteria, and an *Endamoeba*-type nucleus (permanent stained smear). **22**
         f) Trophozoites less than 10 μ in size with *Endamoeba*-type nuclei (stained wet mounts or permanent stained smears). **23**
         g) State any other character or set of characters which you would accept as conclusive proof of *E. histolytica* trophozoites. Indicate type of preparations necessary to reveal characters. **24**
      2) *Presumptive Evidence in Need of Further Confirmation* (i.e. probably *E. histolytica* trophozoites but not absolutely certain)—If you *agree* with any of the following statements, it will be interpreted that you consider the characters as presumptive evidence of *E. histolytica* trophozoites in need of further confirmation. If you *disagree*, please state if you consider the characters as conclusive proof *or* as no significant evidence.
         a) Trophozoites in a warm specimen exhibiting progressive locomotion (saline smear). **25**
         b) Trophozoites with invisible nuclei and with no apparent cytoplasmic inclusions (saline smear). **26**
         c) Trophozoites exhibiting a "slug" shape but with no visible locomotion (saline smear). **27**
         d) Trophozoites forming clear (ectoplasmic), finger-shaped pseudopodia, but without progressive locomotion (saline smear). **28**
         e) Trophozoites with *histolytica*-type nuclei but containing bacteria (permanent stained smear). **29**

f) Trophozoites with *coli*-type nuclei but with no cytoplasmic inclusions (permanent stained smear). 30

g) Trophozoites containing a *coli*-type nucleus and red blood cells (permanent stained smear). 31

h) State any other character or set of characters which you would consider as presumptive evidence of *E. histolytica* trophozoites. Indicate type of preparations necessary to reveal characters. 32

g. *Cystic Stage of E. histolytica*

1) *Conclusive Proof*—If you disagree with any of the following statements, please qualify your answer by giving your evaluation of the characters cited.

a) Cysts less than 10 microns in diameter containing rod-shaped chromatoid bodies with smooth, rounded ends (saline or permanent stained smears). 33

b) Cysts larger than 10 microns containing one to four *Endamoeba*-type nuclei and rod-shaped chromatoid bodies with smooth, rounded ends (combination saline and iodine, or permanent stained smears). 34

c) Cysts containing four *Endamoeba*-type nuclei, but no chromatoid bodies (combination saline and iodine, or permanent stained smears). 35

d) Cysts, regardless of size and number of nuclei, with rod-shaped chromatoid bodies with smooth, rounded ends (combination saline and iodine, or permanent stained smears). 36

e) State any other character or set of characters which you would accept as conclusive proof of *E. histolytica* cysts. Indicate type of preparations necessary to reveal character. 37

2) *Presumptive Evidence in Need of Further Confirmation* (i.e. probably *E. histolytica* cysts but not absolutely certain)—If you *agree* to any of the following statements, it will be interpreted that you consider the characters as presumptive evidence of *E. histolytica* cysts in need of further confirmation. If you *disagree* please state if you consider the characters as conclusive proof or as no significant evidence.

a) Cysts each having a single *Endamoeba*-type nucleus (iodine and permanent stained smears). 38

b) Cysts larger than 10 microns in which neither nuclei nor chromatoid bodies are visible (saline smears). 39

c) State any other character or set of characters which you would consider as of presumptive evidence of *E. histolytica* cysts. Indicate type of preparations necessary to reveal characters. 40

2. *Identification of Organism in Culture*

a. There are no strictly selective media that will support growth of *E. histolytica* only; and it is, therefore, necessary to differentiate cultured organisms by morphological characteristics. 41

b. The food ingested by amebae in culture cannot be used to identify species. 42

c. In culture *E. histolytica* may be motionless and *E. coli* may exhibit progressive locomotion. 43

d. Due to the wide range in variation, the nuclear morphology is not adequate to differentiate *E. histolytica* and *E. coli* trophozoites in culture. 44

e. Whenever possible encystation should be attempted, and the organism identified by the morphology of the cysts. 45

f. *Conclusive Proof of* E. histolytica *in Culture*—If you disagree with any of the following statements, please qualify your answer by giving your evaluation of the characters cited.

1) Majority of trophozoites exhibiting progressive locomotion (saline smears)

and with *Endamoeba*-type nuclei (temporary wet or permanent stained smears). 46

2) Cysts with rod-shaped chromatoid bodies with smooth, rounded ends (saline or permanent stained smears). 47

3) Cysts with four *Endamoeba*-type nuclei (iodine or permanent stained smears). 48

4) State any other character or set of characters which you would accept as conclusive proof of E. histolytica in culture. Indicate type of preparations necessary to reveal characters. 49

g. *Presumptive Evidence of* E. histolytica *in Culture in Need of Confirmation* (i.e. probably *E. histolytica* but not absolutely certain)—If you *agree* to any of the following statements, it will be interpreted that you consider the characters as presumptive evidence of *E. histolytica* in need of further confirmation. If you *disagree*, please state if you consider the characters as conclusive proof *or* of no significant evidence.

1) Easily cultivated (isolation and maintenance). 50

2) Sluggish trophozoites with *Endamoeba*-type nuclei (stained wet mounts or permanent stained smears) which are invisible in saline preparations. 51

3) Cysts having single *Endamoeba*-type nuclei (iodine or permanent stained smears). 52

4) Cysts larger than 10 microns with invisible nuclei (saline smears). 53

5) State any other character or set of characters which you would consider as presumptive evidence of *E. histolytica* in culture. Indicate type of preparations necessary to reveal characters. 54

3. *Collection of Specimens*

a. The normally passed stool is a desirable specimen for an initial examination, due to the ease of collection and the increased likelihood of finding cysts in infected persons. 55

b. If only one examination per person is practical, administration of a cathartic (except oils and milk of magnesia) is advisable since more infections are revealed. 56

c. Cathartic specimens should be examined for *cysts* as well as for trophozoites. 57

d. Since it is not always possible to predict which specimen (1st, 2nd, 3rd, etc.) after catharsis will be most desirable for recovering the organisms, several should be collected. 58

e. If only one specimen can be examined after catharsis, the soft (mushy) passage should be selected since it is likely to contain cysts well as trophozoites. 59

f. The sediment of mucos in a dilute specimen obtained by a saline enema or by a thorough purgation (water specimen) may reveal trophozoites of *E. histolytica* which otherwise may be difficult to find. 60

g. The examination of material obtained by the rectal swab, N.I.H. cellophane swab, cellulose tape, etc. has no demonstrated value in the diagnosis of *E. histolytica*. 61

h. Although the gloved finger used in rectal examinations may obtain material positive for *E. histolytica*, this procedure does not constitute an adequate examination if organisms are not found. 62

i. Granting that proctoscopy (or sigmoidoscopy) is a valuable clinical diagnostic procedure, it should not be relied upon primarily to provide specimens for microscopic examination; and for the purpose of obtaining material for examination, it should be utilized only after other methods have failed to reveal *E. histolytica*. 63

j. Material extruded or aspirated from abscesses suspected of being amebic should

be examined for *E. histolytica* trophozoites, although the organisms are seldom obtained from even confirmed amebic abscesses. 64

k. The aspiration of abscesses should not be used as a primary method of obtaining materials for demonstrating the presence of *E. histolytica*. 65

l. Have you any other type of specimen or method of collection that you prefer for diagnosing intestinal amebiasis? 66

4. *Storage and Chemical Preservation of Specimens*

Although due to the fragile nature of trophozoites, it is highly desirable to examine specimens immediately after passage, it is recognized that this is not always practical and that storage or preservation may be necessary.

a. If an immediate examination is impossible, under no condition should the specimen be artificially heated or placed in incubator, water bath, etc. 67

b. If it is necessary to store the specimen for a few hours, it should be maintained at room temperature or placed in the refrigerator for longer periods. 68

c. Temperatures lower than 4° to 6°C. (i.e. deep freeze) have not proved satisfactory for storage of trophozoites or cysts. 69

d. If the examination has to be delayed 4 hours or more (i.e., during shipment) a portion of the specimen should be mixed with polyvinyl alcohol (PVA)-fixative in order to preserve the trophozoites. 70

e. In view of the relatively prompt postal service within the United States, it is unnecessary to preserve specimens chemically (in formalin, alcohol, etc.) in order to recover and identify protozoan cysts. 71

5. *Examination Techniques*

a. In the identificaton of organisms found in wet smears (saline, iodine, etc.), the oil immersion objective should be employed frequently since it will often reveal structures not visible at lower magnifications. 72

b. *Direct Saline Smear*

1) The saline smear should be used routinely on all specimens, since it is the most nearly normal type of preparation for all stages of the intestinal parasites, and organisms can be detected (spotted) in it with greater ease than in any other type of preparation (ie. iodine, zinc sulphate, permanent stain, etc.). 73

c. *Aqueous Smear*

1) The aqueous smear prepared with tap or distilled water is helpful in the detection of cysts due to its ability to destroy *Blastocystis hominis*. 74

2) The overnight storage of feces containing cysts in a little tap water may reveal chromatoid bodies which were not visible in the fresh specimens. 75

d. *Iodine Smear*

1) Iodine smear is helpful in the identification of protozoan cysts by making visible the number and type of nuclei present. 76

2) Iodine smears as used for cysts should not be used in the examination of amebic trophozoites since it will distort them. 77

3) Chromatoid bodies are less visible in iodine smears than in saline or aqueous smears. 78

e. *Other Wet Stained Smears*

1) Wet stained smears (i.e. Quensel's, crystal violet- hematoxylin, etc.) especially designed for amebic trophozoites may be helpful in distinguishing *Endamoeba* from the other genera. 79

2) Wet stained smears are not critical enough to be relied upon for differentiating trophozoites of *E. histolytica* and *E. coli* on the nuclear morphology alone. 80

f. *Concentration Techniques*

1) There is no practical concentration technique for the amebic trophozoites. 81

2) Except for special helminthological studies, if concentration techniques are routinely employed, one designed to recover *both* protozoan cysts and helminth eggs should be used, (i.e. zinc sulfate, formalin-ether). 82
3) When very rare cysts are found in wet smears, a concentration technique may assist in the identification by increasing the number of organisms in the microscopic preparation. 83
4) If no organisms are found in wet smears, a concentration should be run in order to recover rare cysts that may be present. 84

g. *Cultivation*
1) All the intestinal amoebae will grow in culture; there is no selective medium that will support the growth of *E. histolytica* only. 85
2) Cultivation may be helpful in diagnosing specimens containing only degenerating trophozoites by making possible the production of more normal progeny. 86
3) In cases suspected of *acute* amebiasis, it is worthwhile to culture loose stools in which *E. histolytica* cannot be demonstrated otherwise. 87
4) If cysts (or trophozoites) cannot be demonstrated in formed stools by wet mounts and concentrations, there is no need to attempt cultivation. 88
5) It is more difficult to establish cultures of *E. histolytica* from cysts than from trophozoites. 89
6) The older the specimens the *more* difficult is the cultivation of cysts present. 90
7) With the exception of certain strains, there is no reliable routine procedure of provoking encystation of amebae in culture. 91

h. *Permanent Stained Smears*
1) It is frequently impossible or impractical (due to time required) to find *E. histolytica* organisms in permanent stained smears. Therefore, if organisms cannot be demonstrated in wet smears and by concentration techniques there is little likelihood of finding any in routine examinations of permanent stained smears of the same specimens. 92
2) Permanent stained smears are helpful in revealing the details of the nuclei and the shape of the chromatoid bodies. 93
3) If a permanent stained smear is prepared, the standard Heidenhain iron alum-hematoxylin procedure is to be preferred to shorter techniques in view of its critical results when properly performed. 94

i. *Selection of "Routine" and "Supplementary" Procedures*—It is recognized that the more techniques that are performed on a group of specimens the greater the number of positives that will be discovered. The law of diminishing returns applies, however, and it is advisable to establish certain procedures as "routine", to be run on each specimen, and others as "supplementary", to be used as needed. Ease of detection is of great importance in the identification of organisms in a specimen and should be given primary consideration in selecting the techniques of examination.
1) The *saline smear* should be a "routine" procedure for all specimens. 95
2) The *aqueous smear* is a "supplementary" procedure. 96
3) The *iodine smear* is a "supplementary" procedure. 97
4) Other wet stained smears (i.e. Quensel's, crystal violet-hematoxylin, etc.) are "supplementary" procedures. 98
5) Concentration techniques (i.e. zinc sulphate, formalin ether, etc.) are "supplementary" procedures. 99
6) Cultivation is a "supplementary" procedure. 100
7) The permanent stained smear (i.e. iron alum hematoxylin, etc.) is a "supplementary" procedure. 101

6. *Examination of Multiple Specimens*
   a. One normally defecated specimen can be expected to reveal about ⅓ or ½ of the infections. **102**
   b. One purge specimen can be expected to reveal over ¾ of the infections **103**
   c. Since sigmoidoscopy is effective only in individuals with lesions in the lower bowel, one specimen obtained by this method is limited in revealing organisms. **104**
   d. Since no one specimen (regardless of type) can be relied upon always to reveal *E. histolytica* in infected persons, the examination of repeated specimens is advisable. **105**
   e. It is impossible to determine with absolute certainty freedom from infection in a patient. **106**
   f. In general, the number of repeated specimens should be governed by the clinical suspicion of amebiasis. **107**
   g. In your experience what is the optimum number of specimens to assure maximum efficiency of diagnosis? **108**
7. *Laboratory Reports*

   The laboratory should report all pertinent information in order that the physician can appraise more accurately the value of the results.
   a. If *E. histolytica* is found in the examination, it should be reported by name only—no distinction should be made between the finding of cysts or trophozoites. **109**
   b. Routinely, no distinction should be made in the report between large and small races of *E. histolytica*. **110**
   c. All other organisms encountered, non-pathogens as well as pathogens, should be reported to the physician. **111**
   d. The report should include information on the type and condition of specimens received by the laboratory (i.e. purge, proctoscopic, unpreserved, preserved, age, etc.). **112**
   e. The report should include information on the techniques used in the examination of the specimen (i.e. wet mounts, concentration, permanent stains, cultivation). **113**
   f. If blood, mucus, pus cell, macrophages, and Charcot-Leyden crystals are encountered, they should be reported. **114**
   g. If the examination fails to reveal organisms, a statement such as "No parasites found" or "N.P.F." should be entered on the report and *not* "Negative". **115**

THE "MIF" STAIN-PRESERVATION TECHNIC FOR THE IDENTIFICATION OF INTESTINAL PROTOZOA

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A recently developed MIF (Merthiolate-iodine-formaldehyde) stain-preservation technic, previously described in a preliminary note (Sapero, Lawless and Strome, 1951), has been further tested and utilized by the United States Naval Medical Research Unit No. 3 at Cairo, Egypt for the past two years. It has shown the following advantages over other commonly used stains: (1) simplicity and low cost of preparation, (2) rapid (almost immediate) wet-fixed staining of both cysts and trophozoites of intestinal protozoa and of helminth eggs, and (3) preservative qualities which allow field, home or hospital ward collections of feces in the freshest possible state, i.e., before degenerative changes occur. Laboratory diagnosis may be delayed as long as six months or more without appreciable loss or deterioration of organisms and cellular exudate. Of particular value, fecal specimens may be collected by untrained persons and may be shipped to a central diagnostic laboratory for identification.

Two technics are described: One concerns the use of the "MIF stain" for direct smear identification of protozoa and helminths in fecal specimens brought to the laboratory; the other, its use as a preservative solution which both fixes and stains specimens collected in the field, on wards or in the home.

METHODS

Direct smear technic, for fecal specimens brought to the laboratory. The ingredients of MIF stain, generally readily available in every laboratory, are: tincture of Merthiolate No. 99, 1:1000 (Lilly), Lugol's solution (5 per cent) and formaldehyde solution (USP). The three ingredients may be used in varied amounts within working ranges which give somewhat different results. The Lugol content may vary from 10 to 15 parts, and the formaldehyde content from 10 to 15 parts, with an accompanying adjustment of tincture of Merthiolate to make up 100 parts. The greater the iodine strength, the more immediate the staining reaction. On the other hand, better detectability of cysts and trophozoites is possible at lower iodine strength.

Since Lugol's solution is unstable, *it should be freshly prepared every three weeks.*

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The following proportions of ingredients are recommended to compensate for the gradual deterioration of the Lugol's solution:

WEEK	LUGOL'S SOLUTION (MERCK INDEX)	FORMALDEHYDE (USP)	TINCTURE MERTHIOLATE NO. 99 1:1000 (LILLY)
First	10.0 parts	12.5 parts	77.5 parts
Second	12.5 parts	12.5 parts	75 parts
Third	15.0 parts	12.5 parts	72.5 parts

One cc. of stain (e.g., using Lugol's less than a week old: .10 ml. Lugol's; .125 ml. formaldehyde; .775 ml. Merthiolate), sufficient for 25 to 30 fecal preparations, is placed in a standard Kahn tube. Place distilled water in a second Kahn tube. Put medicine droppers in each, and support both tubes in a Kahn tube-rack.

Place one drop of distilled water (delivered by a small caliber dropper) at one end of a glass microslide and add a drop of equal size of MIF stain solution. To this mixture add the fecal sample. It is important not to mix an excess amount of feces into the staining solution as poor fixation and staining will result. The completed wet preparation should be thin enough to permit the glass microslide to be tipped on edge without the cover slip sliding. The specimen may be ringed with petrolatum or other sealing materials and examined at once, or later if more convenient. If observation of trophozoite motility is desired, a normal-saline direct-smear preparation may be made on the other end of the same slide.

Collection and preservation of fecal specimens other than those brought directly to the laboratory. For the collection and preservation of specimens for survey studies in the field, on hospital wards or in homes, a modification of the "MIF" stain has been devised. This permits the diagnosis of intestinal protozoa and helminth eggs up to six or more months after collection by placing the fecal specimen directly into a preservative solution which has good staining and fixing characteristics. The specimens may be examined by a technician at a convenient time subsequently with the same ease and accuracy as in the case of smears prepared from feces brought to the laboratory.

Prepare:

(a) a stable stock "MF" solution:

250 cc. H_2O (distilled)
200 cc. tincture Merthiolate No. 99, 1:1000 (Lilly)
25 cc. solution formaldehyde U.S.P.
5 cc. glycerine
480 cc. stock ("MF") solution (store in brown bottle).

(b) fresh Lugol's solution (5 per cent), the same as for direct smears.

The following procedure has proved satisfactory:

(1) Measure 2.35 cc. "MF" stock solution into a standard Kahn tube and stopper with a cork.

(2) Measure .15 cc. Lugol's solution (5 per cent) into a second Kahn tube and close tube with a rubber stopper. The two tubes represent a collection unit.

Immediately upon collecting a stool pour the "MF" solution into the Kahn tube containing Lugol's solution. *The two solutions must not be combined until just a few seconds prior to the addition of the fecal specimen*, otherwise a fine precipitate will form; this reduces staining capacity for parasite objects. Thoroughly stir a portion of feces about twice the volume of a medium-sized pea (about 0.25 Gm.) into the mixed solutions with a applicator stick. It is important not to overload the combined solution with feces. After mixing and fecal sedimentation, the top fecal level should be from 1 to 1.5 centimeters from the bottom of the Kahn tube. Stopper the tube containing the specimen, record pertinent data on label and place in rack until time for examination.

If larger samples of fecal material are desired, such as for teaching purposes or for the larger amounts of feces needed for helminth egg concentration procedures, the same proportions of MF and Lugol's solutions are used in increased amounts. Screw cap vials are useful for such specimens to prevent evaporation. As examples:

	AMOUNT OF FECES	MF SOLUTION	LUGOL'S SOLUTION
(small)	0.25 Gm.	2.35 cc.	0.15 cc.
(medium)	0.50 Gm.	4.70 cc.	0.30 cc.
(large)	1.00 Gm.	9.40 cc.	0.60 cc.

This technic has obvious advantages in simplicity for field collecting. The procedure may be further simplified for conservation of space in field kits by adding the required amount of Lugol's solution to the MF vials from a bottle of stock solution by means of a small bore pipette (calibrated at 0.15 cc., 0.30 cc., or 0.60 cc.) just before the stool specimen is added. The earlier addition of iodine is not feasible as a dense precipitate will form upon standing and the iodine will fail to act satisfactorily.

Helminth eggs as well as protozoan trophozoites and cysts retain their natural characteristics and stain as they do in the direct smears. We have observed that the more common helminth eggs may be subjected to the flotation concentration technic by replacing the supernatant fluid in the MIF vial with saturated brine solution and proceeding as for routine recovery of helminth eggs.

To examine an MIF preserved specimen, draw off a drop of mixed supernatant fluid and feces from the *top* of the sedimented layer with a medicine dropper and place a drop on a glass slide. The size of the drop should just suffice to fill the under surface of the cover slip and should not contain too much fecal material, for films that are too thick are unnecessarily difficult to study. Mix the fecal particles thoroughly by means of a toothpick or applicator stick, crushing any large particles. Cover and examine.

Observations indicate that most species of protozoa and helminth eggs tend to concentrate in the upper layer of the sedimented feces. If further parasite concentration is desired, the MIF vial contents may be mixed thoroughly, and the vial allowed to stand until the coarser fecal particles have had time to sediment. The supernatant fluid is then poured into another tube, centrifuged for

3 minutes at about 1500 r.p.m. and a sample of the top sedimented layer of fecal sediment selected for microscopic examination.

Staining reactions. The staining reaction in both direct smear and vial specimen procedures occurs in two phases: (1) an initial iodine stage in which cysts and trophozoites stain from a yellowish green to a yellow brown and (2) a subsequent eosin stage which gradually replaces the iodine and persists. Nuclear and cytoplasmic elements vary in staining intensity with species, age and stage of the protozoan parasites. Fecal débris stains more deeply brown than parasites, blood and tissue cells, etc. No distortion occurs. Helminth eggs also stain and remain morphologically characteristic. At the completion of the iodine phase of staining, the cytoplasm of trophozoites and of immature cysts of all species takes the eosin stain. Nuclear membranes appear to stain dark red to jet black, while nuclear chromatin remains essentially unstained as in the simple iodine stain. Usually all mature cysts also take the eosin stain but in some preparations a few are refractory and will not stain until after several months. This delay in eosin staining of mature cysts of certain species is compensated by the fact that immediate identification is nevertheless possible by morphological characteristics brought out through the fixation process.

The iodine phase of staining is not observed in vial-preserved specimens as it has generally terminated during the time elapsing prior to examination. However, the iodine-staining reaction may be obtained again, if desired, by mixing a small amount of feces from the vial specimen with a fresh drop of MIF as recommended for direct smear use.

Nuclear elements of all human protozoan species except *Dientamoeba fragilis* are well defined. This species may usually be identified by the rather characteristic cytoplasmic detail and the fact that there is an indistinctness or absence of definable nuclei seen in all other species. Flagella are readily detected on all flagellate trophozoites. Extended pseudopods of amebic trophozoites are often observed. Ecto- and endoplasmic demarkation is clearly noted in trophozoites of *E. histolytica*. Glycogen appears as a dark brown area in the iodine phase and colorless in the eosin phase. The halo about the karyosome of both *E. histolytica* and *E. coli* trophozoites may be observed. Chromatoid bodies when present in the cytoplasm appear characteristic, especially in the eosin phase. In trophozoites from cases of amebic dysentery, red blood cells are even more readily defined than in fresh saline preparations. Stages of red blood cell digestion can be differentiated. Ingested bacteria appear distinct in the vacuolated cytoplasm of *E. coli* trophozoites. Charcot-Leyden crystals are easily detected.

For laboratory workers who are just becoming acquainted with the microscopic diagnosis of parasite objects in the stools, the following information will probably be helpful. Preparations from either the direct smear or vial specimen are examined using the 10× ocular and the 16 mm. objective. Helminth eggs and cysts or trophozoites of protozoa measuring more than 10 microns, with a little practice are easily detected at this magnification. With continued practice, even organisms measuring only 8 microns in diameter can be located. For sharper detail one should use the 5× ocular and an 8 or 4 mm. dry objective; for minute

differentiation, the oil immersion objective and 5× or 6× ocular. With the high dry power chromatoid bodies are often visible in *E. histolytica* cysts. For good contrast between cytoplasm and nucleus and for staining nicety, it is important to *use a blue sub-stage filter or a blue filter on the microscope lamp.*

EXPERIMENTAL EVALUATION AND DISCUSSION

Comparative studies on different commonly used smear technics. Although it is planned to present details of the results of a two-year experimental evaluation of the stain-preservative technic in a separate report, a brief summary of the findings is presented here. Identification of saline, iodine, iron hematoxylin and MIF smears (and vial specimens) from identical fecal specimens has been undertaken to determine the relative usefulness of each of the procedures for routine diagnostic work. A majority of fecal specimens examined were heavily populated with both cysts and trophozoites and represented multiple species infections.

Briefly, saline and iodine smear preparations, though simple and rapid, left much to be desired for definitive diagnosis. The iron hematoxylin smear gave excellent results with respect to accurate diagnosis, but at a cost of time and effort, plus technical complexity. In contrast, the MIF technic combined simplicity with definitive diagnosis. It is emphasized that, while these conclusions held well in general, there were instances in which each type of smear preparation provided information of the utmost value.

An important point is that almost without exception examination of the MIF smear alone gave as complete and accurate species diagnosis as did any one or a combination of the three other types of smears. The results with iodine or saline smears alone fell far short in definitive diagnosis. For diagnosis of protozoa the iron hematoxylin smears alone, on the other hand, gave results equal to those of the MIF smears save for flagellate trophozoites, large numbers of which failed to become fixed or were unidentifiable on iron hematoxylin smears. The results clearly indicate that if, as a routine, only a single smear examination is feasible in a busy laboratory, the MIF smear will provide completeness and accuracy of diagnosis with simplicity not obtainable by any other technic. The problem of the desirability of simultaneous detection of helminths has not proved a drawback, except perhaps in specimens containing small numbers of helminth parasite objects, in which case larger amounts of feces (i.e., more than .25–1.0 Gm.) may be required.

Shipment of vial specimens. To test whether shipment of vial specimens through the mail would render specimens undiagnosable, some twenty vials containing numerous species in both cyst and trophozoite stage were mailed to the United States (via air) from the Cairo laboratory. The results reported by the recipients were in close, and usually exact, agreement with the Cairo diagnoses. A series of six such vial specimens, some of which had aged up to five months, were sent to seven different observers known to be expert in the diagnosis of protozoa. Aliquot portions were kept in the Cairo laboratory. The original Cairo results, obtained before shipment, were confirmed. Furthermore, the experienced workers' results were in essential agreement with one another and with the

Cairo results, although none of the U. S. observers previously had had experience with the staining reaction. An exception was a specimen with trophozoites with ingested red cells from a case of acute amebic dysentery. Two observers missed this diagnosis. Re-study of this vial at the Cairo laboratory showed the organisms to be in clumps in the vial, and that there was a reasonable chance of their being missed by failure to occur on a given single smear, or by failure to examine carefully the entire smear. While no appreciable deterioration or loss of organisms was observed after handling incident to prolonged shipment, it is difficult to be certain whether minor loss or deterioration may not have taken place. Further testing on this point is indicated, but so far these difficulties have not occurred to the extent of interfering with diagnosis in the well-populated specimens studied.

Effect of aging. Comments made in regard to the effects of shipment on specimens likewise apply as regards aging. Some deterioration of individual organisms appears to take place gradually during months of preservation, but in the heavily populated specimens tested, diagnosability remained unaltered for six months and even longer. Further evaluation after more prolonged periods of aging is underway.

Home and ward collections, field surveys and other uses. In our experience it has proved advantageous both for laboratory and patient to send in feces collected in the home and mailed or brought to the laboratory in vials. The same has held for specimens collected in actual trials in clinical wards and in field surveys. In all of these, untrained persons have been able to submit satisfactory specimens in fresher state of preservation than has been feasible in our experience by any other previously described method. Furthermore, there is no need for the technician to stop work and to examine a stool immediately in order to obtain the advantage of seeing organisms in their normal state. For the technician who has been unable to obtain and examine freshly passed specimens, the MIF procedure sheds a new light on the morphology of organisms as they appear before degenerative changes set in.

The simplicity of the method suggests that stool surveys in remote parts of the world may be made with little effort or skill required at the point of collection, and with diagnostic study at leisure in a laboratory far distant from the region of the collection.

Another use which we have tested is the collection in vials of relatively large amounts of feces from areas such as in Egypt where a profusion of protozoan forms is obtainable from almost any rural individual. Such specimens can be mailed and are useful for instruction in teaching institutions. Wet mounts on individual microslides may be made. These, when carefully sealed, have been observed still to be in good condition for teaching and demonstration purposes after seven months.

It is also of interest to report that as an experiment a technician who had never before examined stools was taught identification of protozoa solely by the MIF technique in a relatively short time. His accuracy of diagnosis, though having learned and used only a single technic, compared very favorably with the

results obtained by an experienced senior technician employing all the usual procedures of saline, iodine and iron hematoxylin, as well as the MIF method.

SUMMARY

A simple, stain-preservative solution for intestinal protozoa is described. Its use permits busy clinical laboratories to achieve highly accurate stool diagnoses with far less time and technical complexity than is ordinarily required by methods presently in use. A further use is that, if local laboratory diagnostic facilities are not available or if diagnosis is in doubt, fecal specimens may be mailed to a central laboratory for diagnosis or for confirmation of presumptive diagnosis. The technic extends the possibility of satisfactory stool diagnosis to other than the relatively few laboratories in which time and technical expertness permit the very definite advantage of employing all of the known technics for identification of intestinal protozoa.

REFERENCE

SAPERO, J. J., LAWLESS, D. K. AND STROME, C. P. A. 1951, An improved iodine-staining technique for routine laboratory diagnosis of intestinal protozoa, *Science* **114**: 550–551.

OBSERVATIONS ON *PLASMODIUM HUFFI* MUÑIZ, SOARES, AND BATISTA

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INTRODUCTION

The known species of avian malaria fall into two main categories according to the distribution of their exoerythrocytic stages among the various types of host cells. Those in which the exoerythrocytic stages occur predominantly in the lymphoid-macrophage system and in true endothelial cells are of the *gallinaceum*-type and are represented by *Plasmodium gallinaceum*, *P. lophurae*, *P. cathemerium*, *P. relictum*, *P. fallax*, *P. circumflexum*, *P. hexamerium* and *P. durae*. *P. elongatum*, on the other hand, has been until recently the unique representative of another category in which the exoerthyrocytic stages occur predominantly in the erythroblastic series, although other cell types are less frequently invaded. Porter (1942) designated this latter category the *elongatum* type. A new species, *P. huffi*, described by Muñiz, Soares, and Batista (1951) appears to belong to this *elongatum*-type. These authors have kindly sent me blood smears and sections of the organs of infected toucans in which this species of parasite was discovered. Upon basis of study of these preparations I should like to record the following observations.

STAGES IN THE PERIPHERAL BLOOD

Since my observations were limited to a few blood smears, tissue smears from bone marrow, liver and spleen and sections of bone marrow and spleen, I necessarily have incomplete knowledge of the parasite. However, in spite of this limitation, certain conclusions may be reached.

Muñiz, Soares, and Batista described the erythrocytic stages of *P. huffi* as occurring primarily in mature erythrocytes but indicated that some had been seen in polychromatophil elements. I have confirmed the observations on morphology and staining of these forms as described and illustrated by them in their colored plate. One's first impression upon looking at the blood smear is that the parasite belongs to the group of small avian parasites represented by *P. vaughani* and *P. hexamerium*. Since Dr. R. D. Manwell of Syracuse University is especially well informed on this group I sent the blood smears to him for study. His opinion was that there is some possibility that the toucan had a mixed infection of *P. nucleophilum* and another previously undescribed species. Muñiz, Soares and Batista stated that during the acute stage of the infection segmenters with 6 to 8 nuclei predominate whereas in chronic infections the number of nuclei may be 20, 30 or more. In the blood smears which I examined

[1] The opinions and statements contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

the predominant type of parasite belonged in the former category. These facts, together with the morphology of many of the erythrocytic forms, place a doubt upon the purity of the strain described by the Brazilians.

A few parasites were observed in lymphocytes of the circulating blood. These were, however, very sparse in the smears which I examined. The Brazilian authors indicate that thrombocytes, lymphocytes, granulocytes and macrophages of the circulating blood were parasitized. The parasites illustrated (their Plate 2) resemble but are not identical with similar stages of *P. elongatum* in these types of cell.

STAGES IN FIXED TISSUES

The Brazilian authors gave the percentages of host cell types infected by exoerythrocytic stages for bone marrow, spleen, liver and lung. In the bone marrow the erythroblasts and erythrocytes constituted 37.6 per cent of the infected cells; the myeloblasts and myelocytes 40 per cent; thrombocytes 10.8 per cent; macrophages 10.8 per cent and "megakaryocytes" 0.8 per cent. Although the material available to me was not sufficient to permit careful counts I got the impression from the sections of bone marrow which I examined that the lymphoid elements predominate among the cells infected. All asexual stages, including mature schizonts, were seen in lymphocytes. As with *P. elongatum*, these schizonts appeared to remain spherical in shape but, unlike *elongatum*, the numbers of merozoites produced was not as much restricted. It was estimated that 75 to 100 merozoites occurred in one segmenter, and as many as 5 segmenters were seen in one host cell. Some infected myelocytes, thrombocytes and macrophages were observed. However, I did not see many erythroblasts with parasites, as indicated by Muñiz, Soares and Batista. The host cell nuclei were pycnotic and degenerated in most of the infected lymphoid cells by the time the parasite had reached the large schizont or segmenter stage. In some of these infected cells the nucleus appeared very much lobed or polymorphonuclear. I am of the opinion that these malformed cells may have been mistaken by the Brazilian authors for megakarocytes which are not known to occur in birds.

In the spleen it also seemed to me that the preponderance of infected cells were lymphocytes or cells which had originally been lymphocytes but which had been altered by the parasites. Some myeloid elements and macrophages were observed to be infected, but—as in the bone marrow—I could not confirm the finding of the Brazilian authors that a considerable percentage of erythroblasts were infected.

DISCUSSION

It is clear that Muñiz, Soares, and Batista have discovered a very interesting parasite of the toucan, *Ramphastos toco*. Whether the infections they described were due entirely to their new species, *Plasmodium huffi*, or to a mixed infection with this organism and a *Plasmodium* resembling *nucleophilum* is impossible to determine at this time. They were unable to maintain the strain in the laboratory

for lack of a suitable experimental animal. It is very probable, however, that the parasite inhabiting the bone marrow and spleen is new. Since the exoerythrocytic stages of *P. nucleophilum* are unknown, one might ask whether this parasite in some hosts might present an appearance like the one found in the toucan. However, *P. nucleophilum* appears to belong to the group of avian species to which *P. hexamerium*, *P. rouxi*, and *P. vaughani* belong. Manwell (1951) has shown that the exoerythrocytic stages of at least one of these species, i.e., *P. hexamerium*, are such as to place them in the *gallinaceum*-type. Regardless of whether the host cell preferences are better represented by the percentages of different cell types infected which were given by the Brazilian authors or by the impressions which I gained from my incomplete study I believe that this new parasite belongs more nearly to the *elongatum*- type than to either the *gallinaceum*- or *mexicanum*-type of exoerythrocytic development. The cell preferences of *P. mexicanum* are almost exactly a composite of the tendencies shown in the *elongatum* and *gallinaceum* types (Thompson and Huff, 1944), whereas *P. huffi* appears to prefer the blood-forming elements. It should be noted that although *P. elongatum* may be found in the reticulo-endothelial cells it has never been shown to invade true endothelium as is true of the *gallinaceum*-type. *P. huffi* apparently does not invade true endothelium and has no greater preference for reticulo-endothelial cells than does *P. elongatum;* therefore, I believe that this species falls in the *elongatum* rather than the *mexicanum* type as indicated by Muñiz, Soares, and Batista.

Not only does *P. huffi* appear to fit best in the category of parasites with *elongatum*-type of exoerthrocytic stages but it also appears to resemble the species, *elongatum*, in (1) the spherical form of the schizont, and (2) its self-limitation of size in spite of living in cells large enough to permit it to grow larger (Porter and Huff, 1940). It differs from *elongatum* in (1) producing a much larger number of merozoites in the exoerythrocytic stage and (2) in producing abnormalities in certain of the infected host cells. The latter characteristic resembles, although in much lesser degree, the characteristic shown by the schizonts of *Leucocytozoon* (Huff, 1942) which bring about a hypertrophy of the nucleus of the infected cell of the order of 100 times the original size. This characteristic has not been noted in other species of *Plasmodium*.

SUMMARY

Observations on preparations of the toucan, *Ramphastos toco*, infected with *Plasmodium huffi* Muñiz, Soares and Batista indicated the possibility that this infection may have been a mixed one. The exoerythrocytic stages are predominantly of the *elongatum*-type. They are similar to *P. elongatum* in the spherical form and self-limitation in size of the schizonts. They differ from *elongatum* in producing a larger number of merozoites and in effecting abnormal changes in some infected host cells.

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DEVELOPMENT OF *PLASMODIUM BERGHEI* IN *ANOPHELES QUADRIMACULATUS*[1]

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Investigators in this country (personal communications) and abroad (Rodhain and Vincke, 1952; Singh, *et al.*, 1951) have reported difficulty reproducing the results obtained by Yoeli and Wall (1951, 1952) in infecting laboratory-reared anophelines with *Plasmodium berghei* These difficulties occurred even when they used the same species of *Anopheles* and the same strain of *P. berghei* showing abundant gametocytes and exflagellation. Rodhain and Vincke obtained only scanty immature oocysts in 2 of 65 *Anopheles maculipennis* var. *atroparvus* using the SP 28S Keiberg strain, while Yoeli and Wall obtained infection in 18 out of 29 *A. maculipennis* var. *atroparvus* using this same strain of *P. berghei*. As a source of infecting blood meals, Rodhain and Vincke were using the host from which *P. berghei* was originally isolated, *Thamnomys surdaster*, while Yoeli and Wall used the golden hamster, *Mesocricetus auratus*. Splenectomized cotton rats were used unsuccessfully by Rodhain and Vincke in attempts to infect mosquitoes, and Yoeli and Wall reported that they were unable to infect mosquitoes using white rats as hosts for the *Plasmodium*. Singh *et al.* (1951) did not obtain oocysts or sporozoites in 1,832 *Anopheles annularis* fed on mice, rats and garden squirrels with *P. berghei*. Other species of mosquitoes tried by these investigators (*Anopheles stephensi*, *A. subpictus*, *A. jamesi*, *A. ramsayi*, *Aedes* sp. and *Culex fatigans*) were also negative for oocysts and sporozoites with the exception of a single *A. stephensi* which had sporozoites in the glands. We are reporting here the success of our preliminary attempts to infect *A. quadrimaculatus* with the Kasapa strain of *P. berghei* in white mice.

The Kasapa strain was obtained through the kindness of Dr. G. Robert Coatney at the National Institutes of Health. Gametocytes are plentiful and exflagellation can be demonstrated in this strain, while the original KBG 173 strain of *P. berghei* has apparently lost its gametocytes through repeated blood passages and we were never able to infect *A. quadrimaculatus* with this strain. The white mice used in the experiments were all Carworth Farms strain CF 1. The *Microtus* used was reared by Tumblebrook Farms. Our colony of *A. quadrimaculatus* has been maintained in our insectary for several years and was originally obtained from Dr. Richard W. Fay, Savannah, Georgia. Mosquitoes were dissected at intervals from the 4th through the 21st day after an infective blood meal.

Our results indicate that oocysts can be obtained with ease when the mosquitoes are fed in the early stages of blood-induced infection in white mice (Table I). In this rodent, the parasitemia invariably rises steadily until it causes the death of the animal. The parasitemia was always quite high at the time the

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TABLE I

Results of feeding of Anopheles quadrimaculatus *on rodents infected with* Plasmodium berghei, *Kasapa strain*

RODENT HOST	TIME BETWEEN HOST INOCULATION AND MOSQUITO FEEDING	MOSQUITOES		
		Number dissected	Per cent with oocysts	Incubation temperature
White mouse #1	2 days	9	100	21° C.
White mouse #2	2 days	4	100	26° C.
White mouse #3	2 days	7	100	26° C.
White mouse #3	3 days	8	100	26° C.
White mouse #4	3 days	3	100	21° C.
White mouse #4	4 days	23	74	21° C.
White mouse #5	5 days	7	0	26° C.
White mouse #6	5 days	17	0	26° C.
Microtus p. pennsylvanicus #1	4 days	9	66	21° C.
Microtus p. pennsylvanicus #1	7 days	14	0	21° C.

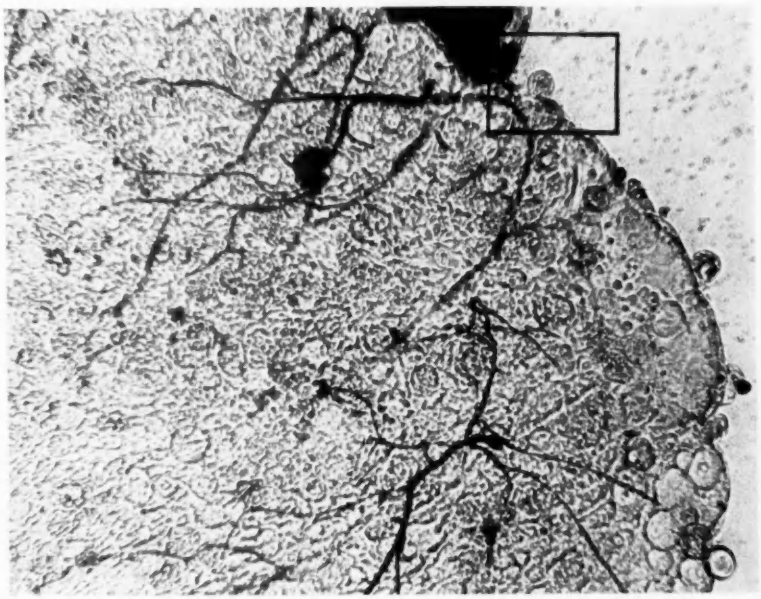

FIG. 1. Stomach of an infected *A. quadrimaculatus* showing oocysts 16 days after a blood meal on a white mouse infected with *P. berghei*. ×140.

mosquitoes were fed; for instance, mouse #1, on the second day of a blood-induced infection, had 155 parasites with one gametocyte per 1000 erythrocytes; while mouse #4, on the third day of its infection, harbored 174 parasites with 4 gametocytes per 1000 erythrocytes. In the *Microtus p. pennsylvanicus*, on the

other hand, the parasitemia reached an estimated 4 to 5 parasites per 1000 erythrocytes on the 4th day and was just barely positive by blood smear on the 7th day (one parasite seen in crossing a blood film once). Mercado and Coatney (1953) have shown that in this rodent, *P. berghei* produces fatal infections in only about one-third of the infected animals, while the remainder recover after an initial parasitemia.

Although we obtained mosquito infections with hundreds of oocysts on the stomach wall, which contained sporozoites as early as the 6th day, sporozoites were never found in the salivary glands, even when dissection took place as late as the 21st day after the infecting blood meal. Figures 1 and 2 show the stomach

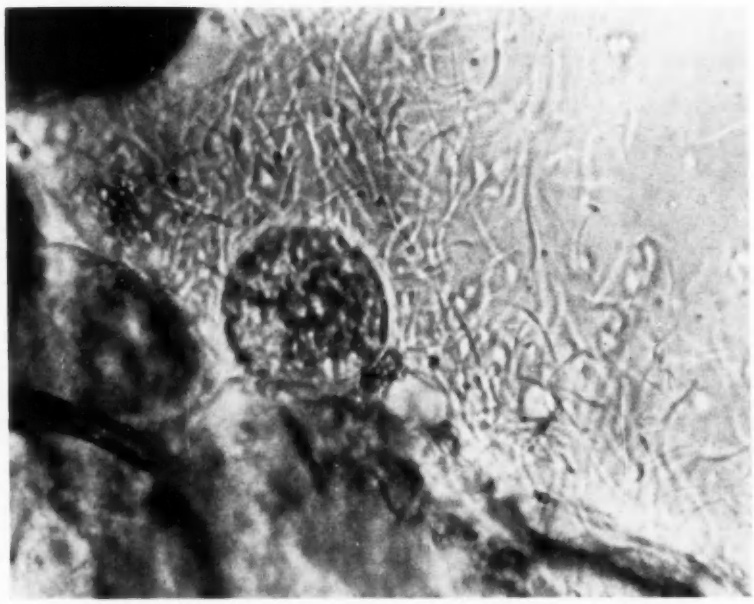

FIG. 2. Portion of stomach shown in Figure 1 (in frame) enlarged to show oocyst and numerous sporozoites which were freed when the stomach was dissected. ×798.

of an *A. quadrimaculatus* dissected after 16 days at 21° C. Although the stomach is surrounded by masses of sporozoites which have broken out of mature oocysts, the salivary glands of this mosquito failed to show sporozoites.

The apparent barrier in the migration of sporozoites to the salivary glands probably indicates that a more suitable laboratory vector should be sought for *P. berghei*, since Yoeli and Wall (1951) also referred to the scarcity of sporozoites found in the salivary glands of their infected anophelines.

SUMMARY

Our results confirm the observations of Yoeli and Wall (1952) that *Anopheles quadrimaculatus* can become infected with oocysts of *Plasmodium berghei*, Kasapa

strain. Two new rodent hosts, the white mouse and *Microtus p. pennsylvanicus*, can be added to those already known to serve as a source of infective meals for anopheline mosquitoes (*Thamnomys surdaster* and *Mesocricetus auratus*).

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CUTANEOUS LEISHMANIASIS IN THE REPUBLIC OF PANAMA

A Report of Twenty-five Cases

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The first case of cutaneous leishmaniasis in the Republic of Panama, was reported by Darling (1910) at a meeting of the Isthmian Medical Association. At the same session, Herrick (1910) reported a second case which was confirmed by Darling. A short time later, Darling (1910a, 1911, 1911a) and Darling and Connor (1911) reported two further cases. Bates (1913) reported the only case with nasal mucous membrane involvement that has ever been observed in Panama. Brosius (1928) reported one case, and in 1930, another one. Kean (1944) reported nine new cases; Johnson (1944) six; and Snow *et al.* (1948) three new ones. Thornburgh *et al.* (1952), have reported their pathological findings in twenty cases, including seven new ones. In summary, then, thirty-two pathologically confirmed cases have been reported to date.

We doubt, however, that this small number of cases represents the true incidence of the disease in Panama, since during the first three months of 1952, we attended seven acute cases and were able to find eighteen cases in the cicatricial or healed stage.

REPORT OF CASES

The first manifestation in all of the seven acute cases was a localized pruritic area of erythema resembling an insect bite. There was no pain or systemic reaction. Five of the patients actually saw an insect biting them, but their descriptions were not sufficiently accurate or detailed enough to identify the insect. A papule which later became a vesicle developed in the center of the erythematous area. The vesicle eventually ruptured due to trauma from scratching and discharged a yellowish serum, forming an ulcer with a granular base and a thickened, elevated, undermined border (Fig. 1).

With ulceration established, the itching disappeared, but local suppuration with crust formation caused varying degrees of pain, directly proportional to the intensity of the inflammation. The diameter of the ulcers varied between two and twelve centimeters. They occurred only on exposed parts of the body (Table 1). No symmetrically localized lesions were noted. The number of the ulcers varied between one and seven. There was superinfestation in three patients due to autoinoculation from the primary ulcer. Acute lymphangitis and regional adenitis due to secondary infection were present in five cases. In four patients the superficial lymphatic vessels were thickened and presented multiple small nodules along their course. These nodules were slightly erythematous, tender, less than one centimeter in diameter and did not ulcerate. There was no case of mucous membrane involvement or systemic reaction.

Three patients showed secondary infection at the first visit, while in the other

four it had apparently been controlled by previous application of local antiseptics. As soon as the secondary infection was controlled, the ulcer's appearance changed markedly, the base became smooth, red and clean, and the borders became flat and thin, losing their undermined character. As the ulcers healed, the lymphangitis subsided and disappeared. The ulcers healed slowly. For a time the scar was pink and slightly scaly (Fig. 2), but later became depigmented, pink, smooth and glistening with a hyperchromic border. It was exactly this latter appearance of the scars that we noted in the eighteen patients examined on our field trips to be described later (Fig. 3).

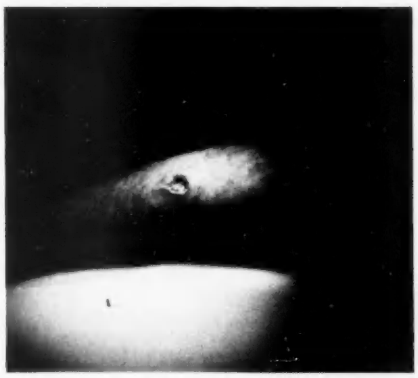

FIG. 1. Ulcer with a granular base and a thickened, elevated, undermined border.

DIAGNOSIS

In all seven acute cases the diagnosis was confirmed by demonstrating *Leishmania brasiliensis* in scrapings and sections from the borders of the ulcers. Smears from the surface of excised nodules of the superficial lymphatics were positive in three of five cases examined. In the eighteen late or cicatricial cases the diagnosis was made taking into consideration the place of residence of the patient, his occupation, the month of onset of the disease, the history of an insect bite, the clinical history of the lesion and negativity of the V.D.R.L. reaction.

EPIDEMIOLOGY

To investigate the surroundings of the seven patients, we visited the villages of Cerro Bandera, Laguna de Chilibre and Mesa de Pacora, accompanied by Drs. M. Hertig and A. G. Fairchild of the Gorgas Memorial Laboratory. We did not go to Arraijan because it has previously been investigated by one of us (C. M. J.) who reported six cases from there in 1944. On these field trips we encountered eighteen cases in the cicatricial stage which will be included in the discussion of the epidemiological data.

A total of twenty five cases were studied, all of the Indo-American race.

TABLE 1

Clinical and Epidemiological Data on 25 Cases of Cutaneous Leishmaniasis in Panama

CASE NUMBER	AGE	SEX	DESCRIPTION OF THE ULCERS				HOW AND WHEN CONTRACTED JUNGLE FARMS				PERIOD OF RESIDENCE YRS.	PLACE OF RESIDENCE
			Number	Stage	Location: Chancre	Location: Super Infestation	Clearing	Sow.	Harv.	Month		
Acute												
1	11	F	7	Ulcerative (3), Scaly-cicatricial (4)	Right arm (3)	Right arm (2)		x		May	2	Cerro Bandera
2	9	F	5	Ulcerative (3), Scaly-cicatricial	Right thigh (3)	Face (2)		x		April	2	Cerro Bandera
3	6	M	2	Scaly-cicatricial	Right thigh (2)			x		May	2	Cerro Bandera
4	29	M	2	Ulcerative	Left knee (1)	Left leg (1)	x		x	July	6	Laguna Chilibre
5	8	F	1	Ulcerative	Right leg (1)					Oct.	7	Laguna Chilibre
6	16	M	2	Ulcerative	Left forearm (1) Left hand (1)		x			Jan.	16	Arraijan
7	34	M	1	Ulcerative	Right wrist (1)		HUNTING			Aug.	Occas.	Messa Pacora
Chronic												
8	24	M	1	Cicatricial	Left leg				x	March	2	Laguna Chilibre
9	19	M	1	Cicatricial	Left leg		x			Jan.	5	Laguna Chilibre
10	36	M	2	Cicatricial	Left leg (1)	Left leg (1)	x			Jan.	4	Laguna Chilibre
11	29	M	2	Cicatricial	Left knee (1)	Left leg (5)			x	July	6	Laguna Chilibre
12	26	M	4	Cicatricial	Right hemithorax (2)	Right hemithorax (2)	x			Jan.	2	Laguna Chilibre
13	36	M	3	Cicatricial	Neck (1)	Right shoulder (1) abdomen (1)	x			March	5	Laguna Chilibre
14	21	F	2	Cicatricial	Right arm (1)	Right hand (1)	x			Jan.	2	Laguna Chilibre
15	36	M	3	Cicatricial	Right leg (2)	Right leg (1)			x	Aug.	10	Laguna Chilibre
16	41	M	1	Cicatricial	Right thigh				x	Aug.	10	Laguna Chilibre

								HUNTING				
17	40	M	2	Cicatricial	Right leg				March	5	Laguna Chilibre
18	30	M	2	Cicatricial	Left leg (1) Left knee (1)			x	Feb.	6	Laguna Chilibre
19	60	M	1	Cicatricial	Right hand		x		May	13	Mesa Pacora
20	25	M	2	Cicatricial	Both hands		x		Feb.	6	Mesa Pacora
21	23	M	1	Cicatricial	Left hand		x		Feb.	6	Mesa Pacora
22	20	F	5	Cicatricial	Left cheek (1)	Both arms (4)	x		Feb.	3	Mesa Pacora
23	26	M	1	Cicatricial	Left leg		x		Feb.	3	Mesa Pacora
24	7	M	4	Cicatricial	Left arm (1)	Right arm (3)	x		July	6	Mesa Pacora
25	22	M	2	Cicatricial	Left leg		x		Feb.	5	Mesa Pacora

(Table 1). The extremes of age were from six to sixty years. There were twenty males and five females. All of the patients, with the exception of the two previously mentioned hunters were farmers who lived in the "bush" and were infected while clearing virgin forest (14 cases), sowing (4 cases) or harvesting (5 cases). The duration of continuous residence in an area before infection occurred, varied between two and seven years. But in all the cases, the cleaning of new bush and the sowing and harvesting of the same coincided with the beginning of the sickness.

The various localities from which the patients came prove that the disease has been observed not in one area alone but in different ones, which justifies the previously expressed opinion of its generalized distribution in the country.

FIG. 2

FIG. 3

FIG. 2. Early cicatricial stage of ulcer, pink and slightly scaly.
FIG. 3. Depigmented, pink, smooth, and glistening scar with a hyperchromic border.

COMMENTS

Since 1910 the existence of cutaneous leishmaniasis in Panama has been amply demonstrated, and it seems strange that only one case of mucous membrane involvement has been reported. In all other respects the clinical manifestations of the disease observed by us corresponded exactly to the well known clinical entity called American cutaneous leishmaniasis.

The epidemiological review of each of the twenty-five cases supports the well known concepts that it is a rural disease, transmitted by an insect vector and acquired during exposure to virgin forest.

The origin of our cases and of those previously reported by other workers in Panama show that the disease is widespread in the Republic with no definite endemic focus.

Finally from the comparatively large number of cicatricial cases discovered on our field trips, we conclude that the disease is by no means rare in Panama and that probably many more cases would be diagnosed if this possibility were con-

sidered in all cases of ulcers of the extremities, and if individuals with ulcerations presented themselves more often for diagnosis and treatment.

SUMMARY

Twenty-five new cases of cutaneous leishmaniasis are reported of which seven were in the acute ulcerative stage and eighteen were in the healed or cicatricial stage. The clinical data obtained agree with those of other American authors with the exception of the rarity of mucous membrane involvement, only one case having been reported so far.

The authors consider that present statistics do not represent the true incidence of the disease in the Republic of Panama and that a better knowledge of the disease will rapidly increase the number of confirmed cases.

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THE FIRST CASE OF HIGHLY DRUG RESISTANT AFRICAN SLEEPING SICKNESS TREATED IN THE UNITED STATES OF AMERICA

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Since tsetse flies (*Glossina oligocena*) disappeared from the Western Hemisphere in the middle tertiary (Cockerell, 1908), outliving the dinosaur (did the dinosaur die of trypanosomiasis ?), there is fortunately no danger of African sleeping sickness spreading in the United States even when a potentially highly infective case harboring plentiful trypanosomes in the blood returns from Africa and settles in this country, as here reported. Nevertheless, there is a real, if remote, possibility that the imported trypanosomes may be transmitted mechanically, for example by tabanids, so that the occurrence of sporadic cases is not excluded. While such a situation still would not imply a public health hazard, the care of the stricken individuals would encounter major difficulties when, as in the present case, the imported trypanosomes are resistant to all classical trypanocidal agents.

The patient, an American (white, male) physician, was infected at the age of 26, in 1929, in the Belgian Congo. Trypanosomes, presumably *T. gambiense*, were present in the blood and lymph glands. At the time of the diagnosis the CSF was not examined, but clinically the central nervous system was not involved. The peripheral trypanosomes persisted after a first treatment with 17 weekly tryparsamide injections totaling 25.5 gm., indicating a strong tryparsamide resistance, but yielded to tartar emetic. The malady progressed insidiously over three years, in spite of continued treatment with tryparsamide and Bayer 205, toward the second stage, the CSF cell count rising from 6 to 20 per cmm., insomnia, drowsiness, headache, excessive tiring after slight efforts and neuritic pains being the main symptoms. An intensive tryparsamide cure, carried through in spite of extreme intolerance, cleared up the symptoms. In 1934 the CSF was normal and the patient was declared cured, having received 93 injections totaling 118.5 gm. tryparsamide, 16.5 gm. Bayer 205 and 3.5 gm. tartar emetic.

During the next four years the patient had no symptoms. In 1938 he returned to the Congo and became reinfected with a highly drug-resistant strain; two weeks after his arrival in an endemic area he came down with fever and had numerous trypanosomes in the blood and subsequently in the lymph glands. After a full course of tryparsamide, trypanosomes were still plentiful in the blood and, after his return to the U. S. A., were persistently present in blood smears taken two years later, after five courses of tryparsamide. Further treatment with 20 weekly i.m. injections of 2 gm. tryparsamide only temporarily improved the situation. As the untoward reactions to each injection became increasingly worse (nausea, vomiting, severe headache) the patient decided to forego further treat-

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ment, opened an office and practised medicine for the next seven years until increasing nervous symptoms led him in 1947 to submit to a full course of pentamidine and in 1948 to a course of 24 injections of Eagle 70 A (p-arsinophenylbutyric acid), causing remarkable, but only temporary relief. Two months after the 70 A treatment, increasingly severe basal headache, neuritic pains and a certain spasticity pointed to another central nervous system flare-up and a CSF examination revealed hypertension and trypanosomes in large numbers. The patient had to abandon his medical activity and became bedridden. His visual field was diminished as a consequence of the tryparsamide treatments. By this time the patient had received since his reinfection 130 injections totaling 140 gm. of tryparsamide, 1 gm. of pentamidine and 24 doses of Eagle 70 A.

In September 1948 the patient was treated with 8 i.v. injections of Mel B (Friedheim, 1949, 1951) (2 series of 4 daily injections, separated by an interval of one week; single dose: .0036 gm./kg). One month after the Mel B treatment the CSF was completely negative, the patient felt better than in years and was able to resume his practice as a surgeon. Two years after the treatment the patient was without symptoms and too busy with a heavy schedule of operations to submit to a CSF control. Four and a half years after the treatment, March 1953, the cure was confirmed by negative trypanosome findings in blood, lymph glands and CSF, and a CSF count of 1 cell/cmm. and 28 mg% protein.

DISCUSSION

The observation covers two distinct consecutive trypanosome infections, both in different degrees resistant to tryparsamide and other drugs.

In the first episode resistance was such that under continued treatment the progress of the disease was merely arrested, and organism and parasite came to a precarious equilibrium lasting five years, until a forced dosage finally eliminated the parasite.

The trypanosome of the second infection was of extreme virulence as shown by the short incubation of two weeks, the rapid involvement of the central nervous system and drug resistance. Nevertheless, a massive tryparsamide treatment achieved a temporary arrest of the pathological process. The extraordinary feature is the length of the period—seven years—during which the equilibrium was maintained without any specific treatment. After seven years the equilibrium was upset in favor of the parasite, which, resistant to tryparsamide, pentamidine and Eagle 70 A, was on the point of defeating the patient by a massive invasion of the central nervous system when it was eradicated by a short course of Mel B. As made plausible by Rollo, Williamson and Lourie (1949), the melaminyl compounds touch on a biochemical system of the parasite which is different and independent of the tryparsamide "receptor", and we may add now, the Eagle 70 A "receptor". Bayer 205 and pentamidine could have no effect in this second stage case because they do not penetrate into the central nervous system.

That the second infection was really a reinfection and not a late relapse is indicated by the fact that after normal CSF findings the trypanosomes appeared first in great numbers in the blood and subsequently in the lymph glands. This

case underscores the known fact that a cured trypanosome infection confers no protection against reinfection and does not mitigate the course of the reinfection in man, as in experimental trypanosomiasis.

SUMMARY

A 24-year case history is reported of two consecutive tryparsamide-resistant second stage trypanosome infections in a white man. The first infection was cured after five years of treatment with 93 injections totaling 118.5 gm. of tryparsamide, 16.5 gm. of Bayer 205 and 3.5 gm. of tartar emetic. The second infection, occurring four years after the first cure, resisted 130 injections totaling 140 gm. of tryparsamide, 1 gm. of pentamidine and 24 doses of Eagle 70 A. After this treatment the CSF contained numerous trypanosomes and the patient was bedridden. A complete parasitological and clinical cure was then achieved by a two weeks treatment with eight injections totaling 1.44 gm. of Mel B, confirmed 4½ years after the treatment by normal CSF findings.

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CARRIÓN'S DISEASE

I. Studies on Plants Claimed to be Reservoirs of *Bartonella bacilliformis*[1]

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Introduction. One of the aspects of Carrión's disease (verruga peruana, bartonellosis) which has not been clarified is the reservoir. From epidemiological considerations it appears that the insect vector, *Phlebotomus verrucarum*, may acquire its infection, at least at times, from sources other than man. The disease may be readily contracted in certain regions which are practically uninhabited and where it is difficult to conceive that sandflies could bite infected persons with sufficient frequency to maintain the infection. On the other hand, Hertig (1939, 1942) discovered a curious infection localized at the tip of the proboscis of sandflies from the verruga zone. These infections affected both sexes equally. While these microorganisms for the most part were not *Bartonella bacilliformis*, nevertheless the latter was recovered in culture on two occasions from the piercing stylets of female sandflies. As Hertig pointed out, the role of these proboscis infections in the transmission of bartonellosis is not clear, but since males do not suck blood, these infections indicate that sandflies have access to substances other than blood which are capable of producing massive local infections of the proboscis.

Carrión's disease is endemic only in well defined "verruga zones" between certain altitudes. In the Province of Huarochirí, Perú, the zone extends from 900 to 2800 meters above sea level (Hertig 1942). It has been recognized for some time that ordinarily bartonellosis is more frequent during the rainy season from December to April (Perez Velasquez, 1914). There is thus a certain correlation between the verruga zones and various types of vegetation, and also between the seasonal incidence of the disease and the period of greatest plant growth. Although these relationships have been observed by the local residents for many years it is only since 1930 that they have received attention from Peruvian investigators. In that year there appeared simultaneously two publications, one by Maldonado (1930) and one by Weiss (1930). The former pointed out the close connection between the incidence of verruga and certain euphorbiaceous plants in parts of the Rimac and Santa Eulalia valleys and suggested the possibility that such plants could be the reservoir of Carrión's disease. Weiss ob-

[1] Studies carried out in the Division of Entomology and Economic Zoology, University of Minnesota, under a fellowship of the John Simon Guggenheim Memorial Foundation.

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served, especially with darkfield illumination, organisms morphologically similar to *B. bacilliformis* in the latex of certain euphorbs from the verruga zone.

There are two species of euphorbs concerned, both small shrubs found on the sparsely vegetated hillsides of the verruga zone, usually in association with cacti and other xerophytes. They are in leaf only during the rainy season. *Jatropha macrantha* (Fig. 1), known locally as *huanarpo hembra* (female), has a thick, smooth, somewhat succulent stem and bears bright red flowers. *J. basiacantha* (Fig. 2), called *huanarpo macho* (male), with a slender woody stem, is armed with small spines and bears white flowers. Both yield a milky latex.

Influenced by the above two publications Mackehenie and associates (1933, 1934, 1935) attempted to clarify the supposed relationships between these plants and bartonellosis. Their results may be summarized as follows: 1. From the latex of the euphorbs, *Jatropha macrantha* and *J. basiacantha*, from the verruga zone, they cultivated a microorganism said to be similar morphologically and pathologically to *B. bacilliformis*. 2. These cultures produced verruga-like nodules in chickens, with bartonellae found both in the red cells and free in the plasma. 3. This organism was agglutinated by serum of verruga patients at dilutions between 1:400 and 1:3200.

The experimental work of Mackehenie and associates has not been repeated by others. Nevertheless the hypothesis that plants were concerned in the transmission of bartonellosis achieved considerable currency in Peruvian medical circles, leading to the suggestion by Escomel (1935) that the suspected plants be systematically destroyed in the verruga zones as a preventive measure. This was actually put into practice on a limited scale in connection with a hydroelectric construction project in the Santa Eulalia valley in 1937. At the same time the natives of the region enthusiastically embraced this plant hypothesis, which acquired something of the status of folklore. In view of the above considerations, together with the confusion which exists in the literature, it has seemed worth while to undertake the experimental studies which form the subject of this paper.

EXPERIMENTAL STUDIES

The principal objectives were to determine (1) whether *B. bacilliformis* could be recovered in culture from plants of the two species of *Jatropha* brought from the Peruvian verruga zone, and (2) whether these two plants could be infected experimentally with cultures of that organism. Since the results in connection with these points were completely negative, there was finally made the attempt to determine up to what point the latex would permit the development of *B. bacilliformis* in culture media currently used for that organism.

1. *Cultures from latex.* With plants sent from the Rimac valley verruga zone to the University of Minnesota, several series of cultures were made from the latex of *J. macrantha* and *J. basiacantha*. These plants were from approximately the same places as those of Mackehenie and Jiménez (1935). The cuttings (stems about 30–40 cm. long) of *J. macrantha* arrived in Minnesota in good condition and soon put forth leaves after being placed in moist soil, which, however, was

not the case with *J. basiacantha* which appeared to have become somewhat dried out during shipment. The culture media were chiefly Noguchi's semi-solid leptospira medium and in a few cases blood agar with 20 per cent rabbit blood.

In making the cultures, the surface of the stem was first washed with distilled water and then with ether. Latex which exuded on making a small puncture with a sterile stylet was transferred by pipette to the culture tubes. Incubation was at 28° C. Two subinoculations were routinely made from each original tube after ten and twenty days. All cultures were observed for at least a month before being discarded. Cultures were made from forty different specimens of *J. macrantha* and twenty of *J. basiacantha* as soon as they arrived at the laboratory. In the case of the former, cultures were made at various times after the plants had leafed out. The results of these series of cultures were completely negative. Contamination occurred in about 15 per cent of the original cultures.

The introduction of latex into the semi-solid media immediately produced such changes as to make subcultures absolutely essential to reveal whether any growth had occurred. Even in the case of first subcultures, especially with *J. macrantha*, further subculture was necessary to observe whether there had been any development of an organism similar to *B. bacilliformis*. Leptospira medium on contact with latex immediately turned dark brown and looked as though it had coagulated. With darkfield illumination there was such a quantity of various sorts of particles that it would be practically impossible to identify *B. bacilliformis* among them.

2. *Experimental inoculations.* Specimens of *J. macrantha* and *J. basiacantha* raised from seed were inoculated in various ways with cultures of *B. bacilliformis*. The seedlings were inoculated 20 to 90 days after germination and varied from about 8 to 25 cm. in height. The youngest had at least three true leaves. The inoculum was obtained from blood agar cultures and was prepared by washing in physiological saline by centrifugation, which gave a suspension free of particles of agar. This suspension was inoculated into the stems or leaves of the seedlings after having washed the selected site with sterile distilled water. In the case of the stems or petioles the epidermis was lightly punctured or scarified so that the bartonellae could come into immediate contact with internal tissues. With the leaves, however, after washing both surfaces, the inoculum was simply deposited on the lower surface or at most the area with the suspension was rubbed lightly with a sterile cotton swab.

Cultures from the latex were begun ten days after inoculation and were repeated periodically for at least three months. The technique was the same as with the older plants from Peru, and the same system of subcultures was followed. A total of 60 cultures was made from 12 inoculated seedlings of *J. macrantha* and 10 of *J. basiacantha*. In no case was Bartonella recovered even though in many cases the latex was obtained from the site of inoculation.

3. *Cultivation of B. bacilliformis in the presence of latex.* In view of the completely negative results of all the foregoing trials, it was desirable to determine whether the microorganism could grow in the presence of latex. Several series of inoculations were made in leptospira medium to which latex had been added

in dilutions of 1:25 and 1:50 by volume. *B. bacilliformis* was recovered from 50 per cent of the 1:50 dilutions with latex of *J. basiacantha* but not from any with the proportion of 1:25. All tubes of both dilutions in the case of *J. macrantha* were negative.

These results indicate that the latex of these two euphorbs offer adverse conditions for the development *in vitro* of the etiological agent of Carrión's disease. Although the cause of this phenomenon has not been determined it may be due to the acidity of the latex. The pH of the latex of *J. macrantha* is about 5.4 while that of the other species is about 6.0. It may be noted that *B. bacilliformis* may be cultivated only between pH 6.8 and 8.4, with the optimum 7.8 (Noguchi and Battistini, 1926).

DISCUSSION

The completely negative results of these attempts both to recover *B. bacilliformis* from the two suspected euphorbiaceous plants from the endemic region and to infect seedlings, together with the failure of the microorganism to grow in the presence of latex in dilutions of less than 1:50 lend no support whatever to the idea that these plants could serve as reservoirs of Carrión's disease. It is indeed difficult to understand the results reported by Mackehenie and his associates, i. e., the recovery from both plants of an organism with the "macroscopic, microscopic and pathologic characteristics of *B. bacilliformis*" (Mackehenie and Coronado 1932, p. 144). Furthermore there is as yet lacking any evidence that there is any close relation between these plants and the insect vector, *P. verrucarum*. The fact that there may exist in the verruga zones certain special types of vegetation is, in the present state of our knowledge, to be regarded as merely one of the climatic-floristic phenomena which characterize the succession of narrow climatic zones on the western slope of the Andes, without any necessary connection with Carrión's disease.

SUMMARY

In testing the hypothesis that certain euphorbiaceous plants may be the reservoirs of Carrión's disease in Peru, the following results were obtained:

1. Cultures of latex of *Jatropha macrantha* and *J. basiacantha* from the Peruvian verruga zone were completely negative for *Bartonella bacilliformis*.
2. Attempts to infect seedlings of both plants with cultures failed completely.
3. The growth of *B. bacilliformis* in vitro is inhibited by the presence of latex in amounts greater than 1 part in 50.
4. There appears to be little likelihood that these plants could serve as the disease reservoirs.

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Explanation of Plate

Fig. 1. *Jatropha macrantha*, known locally as Huanarpo hembra.

Fig. 2. *J. basiacantha*, Huanarpo macho.

These two euphorbiaceous shrubs are conspicuous elements of the sparse vegetation of the mountain valleys in the Peruvian verruga zone, the first with showy red flowers and the other with white flowers. These plants, already established in native folklore as supposed aphrodisiacs, have been claimed by some authors to be the reservoirs of *Bartonella bacilliformis*, the etiological agent of Carrión's disease.

PLATE FIGS. 1 and 2

CARRIÓN'S DISEASE

II. Presence of *Bartonella bacilliformis* in the Peripheral Blood of Patients with the Benign Form

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Introduction. In Carrión's disease there are two well defined clinical phases, hematic and histioid or eruptive (Weiss 1927), represented respectively by the severe anemic form of the disease (Oroya fever) and by the benign eruptive form commonly known as *verruga peruana*. Although adequate studies have not been carried out it has been supposed that *Bartonella bacilliformis* is present in the blood of all patients during the whole of the hematic phase. Furthermore, some authors have held that the organism is constantly present in the blood not only in both phases of the disease but persists for many years afterward (Aldana 1929). It is obvious that to establish this as a constant phenomenon would be of great importance in connection with the possible role of such carriers as reservoirs of the disease.

For over ten years, in the laboratory of the Section of Medical Entomology of the National Institute of Hygiene and Public Health, Lima, the author has routinely made blood cultures from patients with Carrión's disease, and has thus acquired a very considerable experience with this phase of the matter. It has been observed that a considerable proportion of the benign cases yield negative blood cultures and that this is frequent even in the stage preceding the appearance of the eruption. Since *B. bacilliformis* is readily isolated and cultivated by anyone with adequate experience and appropriate culture media, these findings would indicate that this microorganism is not continuously present in the patients' blood. This would naturally impose a limitation on the diagnostic value of blood culture in this disease, a method which at present is considered to be the best and perhaps at times the only means of diagnosing the benign form.

EXPERIMENTAL STUDIES

A combination of circumstances presented the opportunity of verifying under practically experimental conditions the above mentioned observations. In June 1947 the Institute of Hygiene initiated under the direction of the author a series of experiments in the control of Carrión's disease and cutaneous leishmaniasis through a campaign against *Phlebotomus* sandflies with DDT. Part of the experimental project was carried out at the Agricultural School at San Juan, located in the midst of the verruga zone of the Rimac valley, at Kilometer 68 of the Central Highway, and 2200 meters above sea level. Each year classes begin

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the first part of May. Students come from various parts of Peru, in many of which verruga does not occur. Since the students are in residence at San Juan during the entire school year, May to December, and are subject to the school's disciplinary control, we had exceptional conditions for carrying out observations on Carrión's disease.

The first application of DDT was made June 15, 1947, as a residual spray on the inner walls and ceilings and also on the outer walls of all the school buildings,

TABLE 1

Blood cultures to recover Bartonella bacilliformis *from patients with the benign form of Carrión's Disease, San Juan, Matucana, 1947–1949*

CASE NO.	CULTURES MADE IN 1947							REMARKS
	June	July	Aug.	Sept.	Oct	Nov.	Dec.	
	I. *Patients with at least one positive blood culture*							
5	POS				NEG		NEG	Eruption Dec. 1947
25	POS		POS	NEG	NEG	NEG	NEG	Eruption Sept. 1948
29	NEG						POS	No eruption up to Sept. 1949; blood culture neg., May 1949
38	POS	POS	NEG	NEG	NEG	NEG	NEG	Eruption Oct. 1947
45	NEG		POS	NEG	NEG	NEG	NEG	Eruption Dec. 1947
52	POS	POS	POS	POS	POS		Cont	Eruption Oct. 1947
65	POS		POS	NEG	NEG	NEG	NEG	No eruption up to May 1949; blood cultures neg., June and Nov. 1948
76	POS		NEG	NEG	NEG	NEG	NEG	No eruption up to Dec. 1948; blood culture neg. Nov. 1948
83	NEG						POS	Not seen after Dec. 1947
	II. *Patients with verruga eruption and negative blood cultures*							
2	NEG						NEG	Eruption June 1947
12	NEG						NEG	Eruption July 1947
24	NEG				NEG		NEG	Eruption Oct. 1947
35	NEG						NEG	Eruption Dec. 1947
63	NEG		NEG					Eruption Feb. 1948

dormitories, mess hails, classrooms and offices. From our own experience as well as that of others (Hertig 1949) we felt sure that with such an application of DDT there would be little or no danger for at least several months that sandflies could enter and bite persons in the treated rooms and thus transmit verruga. On the same day (June 15) blood smears and blood cultures were made from all students, with the object of determining how many had acquired the disease while living in San Juan without protection from sandflies. Of the 81 students, seven gave cultures positive for *B. bacilliformis*. In one case, in addition to the positive blood culture, the blood smear was positive also. This student was obviously very ill, although he was not in bed. He died a few days later.

The attending physician was not sure whether or not bartonellosis was the cause of death.

Our first plan in connection with the control of verruga had been to make only two blood cultures, one in June and one in December at the end of the school year when the students would be returning to their homes. The results of the first cultures, however, caused the plan to be changed to take advantage of the exceptional material available for study. It was decided to make periodic blood cultures from those students who had been positive in June, and from any others who might exhibit clinical symptoms of verruga. Among other things it was decided to determine for how long a period *B. bacilliformis* could be recovered in culture from the peripheral blood. In this connection we assumed that the students would be sufficiently protected against sandflies so that there was only a remote possibility that they would become reinfected after June 15, 1947. With the enthusiastic and effective cooperation of the Director of the Agricultural School, Ingeniero Carlos Delgado Vivanco, and of the teaching staff, it was possible to carry out this program.

The results of these periodic blood cultures are summarized in the table, together with the data covering five cases in which the infection was revealed only by the appearance of the cutaneous eruption. There are also included two cases, Nos. 29 and 83, which gave positive blood cultures in December. Unfortunately in the case of No. 83 cultures were made only in June and December, after which the student was not seen again. Case No. 29 did not attend the school during 1948 but a culture was made in May 1949, with negative result. The fatal case with both positive culture and blood film is omitted from the table.

DISCUSSION

Diagnostic value of blood cultures. From the table it will be noted that with the sole exception of Case No. 52, all who were positive by blood culture in June gave negative cultures two to four months later, and were consistently negative thereafter, although the subsequent appearance of the eruption in most of these cases showed that they still carried the infection. It will be seen that from the first positive cultures until the appearance of the eruption for cases Nos. 5, 25, 38, 45 and 52, the proportions of positive cultures were 33, 33, 40, 20 and 100 per cent respectively (not counting the contaminated culture of Case No. 52), an average of 45 per cent positive. For Cases Nos. 65 and 76, out of eight and seven cultures, 25 and 14 per cent respectively were positive, but since no eruption had occurred within the 18 to 23 months' period of observation, the duration of their infection remains unsettled.

It follows that the relatively low proportion of positive cultures from persons known to be infected with *B. bacilliformis* considerably reduces the significance of negative blood cultures in attempting to diagnose the benign form of Carrión's disease. This is emphasized by the last five cases in the table (Nos. 2, 12, 24, 35 and 63) which at no time gave positive cultures, even in the presence of persistent symptoms or of the eruption itself.

On the other hand, the table indicates quite clearly that the results of blood

culture depend to a considerable extent on the stage of the infection. The chances of diagnosing the disease are much greater during the first months of the infection and decrease markedly thereafter. This phenomenon should be borne in mind in any experimental studies on immunization or treatment.

Asymptomatic cases. It has been known for some time that it is possible to recover *B. bacilliformis* in culture from residents of the verruga zone who show no symptoms at the time. This phenomenon was first demonstrated by Battistini (1927) and subsequently confirmed by others (Weinman and Pinkerton, 1937; Hurtado, 1938; Herrer and Urteaga, 1943) in various endemic areas in Peru. There is a tendency to consider such asymptomatic cases as possible reservoirs of the disease (Weinman and Pinkerton, 1937; Rebagliati, 1940). These various workers were investigating the prevalence of infections of *B. bacilliformis* in a given area at a certain time, for which a single sample of blood was deemed sufficient. As far as our information goes, in none of the asymptomatic infections was there a second sample of blood tested within a matter of weeks or months. For this reason these investigators had no opportunity of determining how long *B. bacilliformis* might persist in the peripheral blood of their asymptomatic cases.

In our studies at San Juan, the 14 cases of Table 1 may be grouped as follows on the basis of symptoms of Carrión's disease (fever, malaise, bone and joint pains, or eruption):

A. Cases with manifest symptoms: ten (Nos. 2, 5, 12, 24, 25, 38, 45, 52, 63, 83).

B. Cases without appreciable symptoms: four (Nos. 29, 35, 65, 76).

The proportion of asymptomatic cases was thus about 29 per cent. In those cases which presented symptoms, they varied in intensity and in some persisted until the appearance of the eruption. In the asymptomatic cases, the results of blood culture were not markedly different from the series with manifest symptoms.

The negative results of the repeated blood cultures in two of the asymptomatic cases, Nos. 65 and 76, could be interpreted as indicating that *B. bacilliformis* was present in the peripheral blood (a) only transiently on those occasions when positive cultures were obtained, or (b) in numbers so small that it was not possible to cultivate them with certainty. On the other hand, the fact that neither these two cases nor Case 29 developed the eruption within the 18 to 21 months during which they were under observation after their respective positive cultures could also indicate that in such cases the infection itself was only transitory. Whatever the explanation, these findings indicate the necessity for further study of asymptomatic cases.

SUMMARY AND CONCLUSIONS

Blood cultures made over a period of six months from persons known to be infected with *B. bacilliformis*, the etiological agent of Carrión's disease, support the following conclusions:

1. The proportion of positive cultures is much higher in the first months of infection than later.

2. Negative blood cultures have little significance in the diagnosis of the benign form of the disease.

3. The results of blood culture are similar in both asymptomatic infections and those with manifest symptoms.

4. The inability to cultivate *B. bacilliformis* with regularity from the blood of asymptomatic cases reduces the likelihood that these cases can serve as reservoirs of the disease.

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CARRIÓN'S DISEASE

III. Experimental Infection of Squirrels[1]

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During the past fifty-odd years a number of investigators have carried out many experimental inoculations of laboratory animals with *Bartonella bacilliformis*, the etiological agent of Carrión's disease. In the earlier studies the material inoculated consisted of patients' blood, tissue from the verruga nodules and in certain cases suspensions of the insect vector, *Phlebotomus verrucarum*. Following the demonstration of the cultivation of *B. bacilliformis* by Noguchi and Battistini in 1926, cultures have been frequently used as inoculum. Among the animals inoculated have been: (a) monkeys of various species (Jadossohn and Seiffert, 1910; Ribeyro *et al.*, 1914; Hertig, 1942, and others); (b) dogs (Tamayo, 1899; Noguchi *et al.*, 1929; Samper and Montoya, 1940, and others); (c) burros (Ribeyro *et al.*, 1914; Noguchi *et al.*, 1929); (d) guinea pigs (Ribeyro *et al.*, 1914; Samper and Montoya, 1940, and others); (e) rabbits (Strong *et al.*, 1915; Ribeyro *et al.*, 1914); and (*f*) chickens (Aldana, 1929; Weinman and Pinkerton, 1937). In these various investigations the objectives were to reproduce the disease in animals or to determine their susceptibility to *B. bacilliformis*. With almost all the species of monkeys tested (*Macaca mulatta*, *Cebus* spp., *Cercopithecus* sp., etc.) it was possible to produce typical verruga nodules at the sites of intradermal or subcutaneous inoculation and in addition it was frequently possible to recover *B. bacilliformis* in culture from the peripheral blood. With other animals, however, the results were far from uniform and in some cases contradictory.

In spite of frequent reports by natives of the verruga zone that certain domestic animals often present verruga nodules, up to the present such infections have not been demonstrated. No animal of any sort has been found naturally infected with *B. bacilliformis* with one exception. Hertig (1948) cultivated this organism from the blood of a single field-mouse, *Phyllotis* sp., from the Rimac valley verruga zone. Attempts to recover this organism from numerous other individuals of the same and other rodent species from the same region all failed, as did also attempts to infect this rodent with cultures. In view of the unsatisfactory state of knowledge about the susceptibility of animals in relation to the

[1] These studies were carried out in 1949–1950 in the Division of Entomology and Economic Zoology of the University of Minnesota, under a fellowship from the John Simon Guggenheim Memorial Foundation. The author desires to express his warmest thanks to Dr. Clarence E. Mickel, Chief of the Division of Entomology and Economic Zoology, for the many facilities which were placed at his disposal; to Dr. G. B. Fairchild, Professor of Medical Entomology, under whose immediate direction this work was carried out; and to Dr. Marshall Hertig, Gorgas Memorial Laboratory, Panama, for many valuable suggestions and criticisms on various occasions.

problem of potential reservoirs of Carrión's disease, together with the lack of a truly satisfactory laboratory animal, a series of inoculations of small rodents was carried out at the suggestion of Dr. Marshall Hertig, Gorgas Memorial Laboratory, Panama. Particular attention was paid to the possibility that the skin could be infected without showing any external sign and even in the absence of demonstrable organisms in the peripheral blood.

MATERIAL AND METHODS

Strains of B. bacilliformis. We used four Peruvian strains of *B. bacilliformis*, two maintained *in vitro* for about ten years and two recently isolated. The former were originally isolated by Dr. Marshall Hertig, one (strain No. W-372) from a naturally infected sandfly in 1939, the other (No. 529), exact source unknown, at about the same time. The two recent strains (Sv-5, Sv-15) had been isolated from cases with the anemic form of the disease within one and six months and were in their second and tenth passages respectively.

The strains were maintained in semisolid leptospira medium. Cultures in this medium were used for subcutaneous inoculation, but for the intradermal and intravenous routes it was necessary to use blood agar cultures. The organisms were washed once or twice by centrifugation in 0.85 per cent saline to obtain a suspension of bartonellae as free of agar as possible. By this method it was possible to inoculate an animal intravenously with the organisms from two to four culture tubes, with about the same quantity being used also for intradermal injections.

Squirrels. A total of 30 squirrels were used, 18 ground squirrels (gophers), *Citellus tridecemlineatus*, and 12 grey squirrels, *Sciurus carolinensis*. The gophers were captured in Calvary Cemetery, St. Paul, Minnesota, and the grey squirrels on the campus at University Farm, St. Paul. Apparently all thirty animals were adults.

After inoculation periodic blood cultures, and cultures from the skin were made. Skin cultures from the sites of inoculation as well as elsewhere on the body were made as follows: the skin was shaved and then disinfected with alcohol and ether. A bit of skin was cut out with scissors and then triturated in a little saline in a test-tube or petri dish. The final suspension was transferred to the culture tubes.

RESULTS

Sciurus carolinensis. Two of the 12 grey squirrels died as the result of an accident on the day after inoculation and were discarded. Of the remaining ten, three were inoculated intradermally and intravenously, one intradermally and intracardially, one intradermally and subcutaneously, one subcutaneously and intravenously, two intradermally only, one subcutaneously only, one intravenously only. The first cultures were made within five days, usually on the first or second day after inoculation, except in one case when cultures were begun on the ninth day. Two to four subsequent cultures were made at intervals from each animal for 21 to 105 days after inoculation. For the most part the

cultures were in pairs, from the blood and skin, either at the site of inoculation or elsewhere. From five of the ten squirrels it was possible to recover *B. bacilliformis* in culture from one to ten days after inoculation. Of these three gave positive skin cultures from the site of inoculation at four, six and ten days respectively; a fourth gave a positive blood culture on the day following inoculation and positive skin culture from the site of inoculation on the fourth day. The fifth squirrel, while negative by blood culture on the first day, was positive on the tenth. Both squirrels which were positive by blood culture had been inoculated both intradermally and intravenously. All skin cultures made from other than the sites of inoculation were negative. Two animals were inoculated with the old strains (W-372, 529) and were both negative. A third, inoculated intradermally with the recent strain, Sv-5, and intracardially with W-372, was positive by culture from the skin but not the blood on the tenth day. The remaining seven animals (four positive, three negative) were inoculated with one or both of the recent strains. Out of a total of 34 cultures from the blood and 31 from the skin, the only contaminated tubes were three from the skin.

Grahamella-like organisms. In 15 per cent of the blood cultures and 6.5 per cent of the skin cultures, there was recovered a Gram-negative microorganism morphologically somewhat similar to *B. bacilliformis*. In its growth characteristics in leptospira medium it also resembled this organism somewhat, especially in the color, form and grouping of the colonies. Nevertheless, with darkfield illumination it was possible to differentiate this organism with certainty from *B. bacilliformis*. Only three of the ten squirrels (one of them also positive for *B. bacilliformis*) yielded this unidentified organism, which has not been classed as a contaminant. In its morphological and cultural characteristics this organism is very close to the genus *Grahamella* Brumpt 1911. It is well known that these microorganisms parasitize the red cells of various mammals, especially small rodents, and certain species are readily cultivable in leptospira medium and blood agar (Tyzzer, 1942). The parasitized red cells may be extremely scarce and the infection may be demonstrable only by blood culture (Herrer, 1944).

In the case of the grey squirrels, intradermal inoculation produced a marked skin reaction, evidenced after the second or third day by a local induration which later became somewhat prominent and red. In some cases on the seventh or eighth day the indurated areas had attained a diameter of 8–10 mm. and from their prominence and redness somewhat resembled verruga nodules. However, on being punctured there was very little bleeding and in general after the tenth day they began to be resorbed and disappeared several days later. A similar reaction was produced by the inoculation of leptospira medium alone and also with heat-killed cultures of *B. bacilliformis*, which indicates that the reaction is not specific. The skin recovered its normal appearance slowly. In some cases it was a month before the affected areas became newly covered with hair.

Citellus tridecemlineatus. The 18 gophers were each simultaneously inoculated intradermally on the abdomen and intravenously (14) or intracardially (4). Only the recently isolated strains (Sv-5, Sv-15) were used. In the case of 15 of these animals, which included the four inoculated into the heart, cultures were

made four to eight days after inoculation, with a second series of cultures after another 10 to 21 days (14 to 29 days after inoculation). Paired cultures from blood and skin at the site of inoculation were made throughout with two exceptions when blood cultures alone were made. In no case was *B. bacilliformis* recovered. With the remaining three gophers, however, cultures were begun on the day following inoculation and from all three *B. bacilliformis* was cultivated, but only from 24 to 48 hours after inoculation. In two instances cultures from the site of inoculation were positive, one on the first, the other on the second day. The third gave a positive blood culture on the day following inoculation. Subsequent cultures on the third and 23rd day were negative for all three animals. In the total of 38 blood cultures and 39 skin cultures, contamination was encountered only in five from the skin.

Grahamella-like organisms. The gophers were much more commonly infected than the grey squirrels with the *Grahamella*-like organisms. Only one out of the 18 failed to yield them at one time or another. This one gopher was positive for *B. bacilliformis* on the first day. Eighty-four per cent of the blood cultures and 46 per cent of the skin cultures—in the case of eight animals, 100 per cent of all cultures—were invalidated by the growth of these organisms. This constituted a serious difficulty, since in addition to resulting in the elimination of such a large proportion of the cultures and nearly half the animals involved, it was necessary in practically every case to make several subcultures from the original tube, which in turn involved prolonged darkfield examination.

In contrast to the grey squirrels, the gophers showed no appreciable skin reaction to intradermal inoculation. After two or three days the skin appeared normal and was soon covered with new growth of hair.

DISCUSSION

The results of our inoculation of the two species of squirrels differ from those obtained by various authors with animals susceptible to infection with *B. bacilliformis*, such as various species of monkeys, in that it was not possible to produce verruga nodules in the squirrels. The recovery of *B. bacilliformis* from the skin of the grey squirrels up to ten days after inoculation would indicate the possibility that in the endemic region certain animals could have the skin infected without showing any external evidence, either in the form of verruga-like nodules or otherwise. This is of potential importance in the epidemiology of Carrión's disease, since no reservoir has been demonstrated and the matter remains completely open.

SUMMARY

Two species of squirrels were inoculated intravenously and intradermally with cultures of *B. bacilliformis*. This organism was recovered in cultures of the blood and of the skin at the sites of inoculation up to the tenth day in the case of the grey squirrel, *Sciurus carolinensis*, but only up to 48 hours with the gopher, *Citellus tridecemlineatus*. Skin cultures from other than the sites of inoculation were negative. No verruga nodules were produced in either rodent.

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HISTOPLASMIN AND COCCIDIOIDIN TESTING IN A PHILIPPINE TUBERCULOSIS HOSPITAL

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Skin testing to detect present or past infection by pathogenic fungi has received considerable attention in the United States. This procedure has gained much importance in the search for areas of endemicity of histoplasmosis and coccidioidomycosis.

In the past five years individuals in many parts of the world have been tested with histoplasmin and coccidioidin. These are sterile filtrates from broth cultures of *Histoplasma capsulatum* and *Coccidioides immitis* respectively. Reports indicate a comparatively high percentage of reactors in those areas of the world where cases of histoplasmosis or coccidioidomycosis have been found and no reactors or a low percentage of reactors in those countries from which few or no cases have as yet been reported.

Recently Christie (1951) summarized the reports of histoplasmin testing and suggested that climatic factors as well as geographical focus seem to play important roles in the distribution of histoplasmosis. Since both *Histoplasma capsulatum* (Emmons, 1949) and *Coccidioides immitis* (Stewart and Meyer, 1932) have been isolated from soil in the endemic areas of the United States, it is assumed that the focus for infection comes from the soil.

Most histoplasmin surveys have been made along with tuberculin surveys. Since most of the Filipino patients upon whom this study was conducted had demonstrable acid-fast bacilli in their sputa, tuberculin testing was not done. It is well known that tuberculosis is a grave problem in the Philippines. Young (1949) in a tuberculin survey of 5,448 school children in Manila in 1946 found that 48–55 per cent were reactors at the age of 6 years and 100 per cent reactors at the age of 18. In a roentgenogram survey of 127,971 Filipinos in 1947–1948 he reported 6.0 per cent as having tuberculosis and 9.9 per cent as suspected tuberculosis. Bocobo and Reyes (1950) showed that 97.9 per cent of 477 medical and nursing students who represent the higher socio-economic levels in Manila were reactors to tuberculin.

SURVEY

This study was made on 824 patients at Quezon Institute, a tuberculosis hospital in the Manila area, in January 1951. These people, ranging in age from one to 70 years, consisted of 457 males and 367 females. Approximately ten per

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cent were under the age of 16 years. Although the majority of the patients resided in Manila or its neighboring provinces, many of the provinces of the Philippines were represented.

The histoplasmin (Duke Lot # H-42) was used in a dilution of 1:1000 in sterile saline. The coccidioidin (Smith Lot # 29–31) was used in a dilution of 1:100 in sterile saline. An American with known histoplasmin and coccidioidin reactions was used as a control for the skin testing substances used in this survey. Each subject was injected intradermally with 0.1 ml. of the diluted histoplasmin on the volar aspect of the right forearm after the area had been cleansed with 70 per cent alcohol. The left forearm was similarly injected with coccidioidin. At the end of 48 hours, the site of injection was examined for induration with or

TABLE 1

Filipino tuberculous patients tested with histoplasmin and coccidioidin: number of reactors and degrees of reaction

	TESTED WITH HISTOPLASMIN						TESTED WITH COCCIDIOIDIN
	Number tested	Positive reactors		Degree of reaction			Positive reactors
		no.	%	+	++	+++	
Men	457	16	3.76	6	7	3	0
Women	367	10	2.92	3	7	0	0
Total	824	26	3.15	9	14	3	0

without erythema. The degree of reaction was classified according to the following criteria:

Negative.....induration with a diameter less than 5 mm.
+.....induration with a diameter of 5 to 9 mm.
++.....induration with a diameter of 10 to 19 mm.
+++.....induration with a diameter of 20 or more mm.

Each reacting patient was retested two or three times and gave a consistent reaction. Roentgenograms of all patients showed some form of infiltration of the lungs. Acid-fast bacilli were found in the sputa of 22 of the 26 reactors to histoplasmin. This represented 13 men and 9 women.

RESULTS

None of the 824 patients showed a reaction to the coccidioidin.

Twenty-six patients developed a reaction to the histoplasmin. This represented 3.15 per cent of the people tested. Tabulation of the reactions can be seen in Table 1. The positive reactors ranged in age from 16 to 63 years.

DISCUSSION

The Philippines has been incriminated as one of the endemic areas of histoplasmosis by the review of Meleney (1940) who considered a case seen by Strong

(1906) and one by Wade (1926) as histoplasmosis. Histoplasma-like organisms were seen in tissue sections from both of these cases. The paper by Strong even antedates Darling's report of histoplasmosis by several months. Strong also considered the organism seen in tissue section to be a fungus.

Mendoza (1947) reported a case of histoplasmosis. However, this proved to be a generalized *Candida albicans* infection on further laboratory study (Davis, 1952). The fungus, *Histoplasma capsulatum*, has never been cultured from a patient in the Philippines.

Bocobo and Reyes (1950) reported histoplasmin testing on 477 Filipino medical and nursing students. They found 4.61 per cent to be reactors. These results together with the tuberculous patients reported in this paper make a total of 1,301 Filipinos who have been tested with histoplasmin; 3.68 per cent were found to be reactors. This low percentage of histoplasmin reactors further establishes the rarity of histoplasmosis in the Philippines.

In the endemic areas for coccidioidomycosis in California, some Filipinos have been found to develop progressive coccidioidal granuloma with a high mortality rate. Therefore, the absence of reactors to coccidioidin in this survey indicates that coccidioidomycosis is probably not present in the Philippines.

SUMMARY

In this survey, 824 tuberculous patients in the Philippines were tested with histoplasmin and coccidioidin; 3.15 per cent of the patients reacted to the histoplasmin, but no reactors to coccidioidin were seen.

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STUDY OF A RESIDUAL POPULATION OF *ANOPHELES L. LABRANCHIAE* FALLERONI IN THE GEREMEAS VALLEY, SARDINIA[1]

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The island of Sardinia in the Mediterranean Sea has been the site of a large-scale experiment to establish the feasibility of the eradication of an indigenous malaria vector, *Anopheles l. labranchiae* Falleroni. The progress of this project has been reported by Logan (1950) and is to be the subject of a full account edited by Logan (Monographic Series of the American Journal of Hygiene). One of us (T.H.G.A.) made a survey of the anophelines of the island during the summer of 1946 and was present throughout the entire eradication period. The full-scale eradication operation, a joint effort of the Italian Government in cooperation with UNRRA and later ECA, under the technical direction of The Rockefeller Foundation, was carried on from 1947 through 1950. Since 1950 the domestic insect control service of the Sardinian Regional Government has carried on the work as funds permitted.

While throughout most of its range *labranchiae* is a coastal marsh breeder, in Sardinia this mosquito was very generally distributed in the quiet water of streams even at considerable elevations. Thus, for a time during the eradication operation, all water surfaces on the island were treated as actual or potential *labranchiae* breeding places. Later, however, only the places where *labranchiae* was found were subjected to larvicide treatment. At the conclusion of the ERLAAS[3] campaign in 1950 only very few *labranchiae* could be found anywhere on the island. The efforts of the local government since that time have been directed at the elimination of the species from these few remaining foci.

As the eradication effort proceeded, a number of fundamental and perplexing problems emerged. With eradication within a limited time as the prime objective, it was not possible to investigate these problems, which were in many respects wholly new and without precedent, there having been no prior attempt to eradicate so well-established an indigenous mosquito in so large an area of such varied terrain. Of particular interest was the question of how the minute residual population of *labranchiae* would respond to the vacuum created by the eradication effort, once control measures were suspended or reduced. The writers spent much of the active mosquito-breeding season of 1952, from late June through the end of September, in an attempt to resolve certain of these problems.

[1] The studies and observations on which this paper is based were conducted with the support and under the auspices of the Division of Medicine and Public Health of The Rockefeller Foundation with the cooperation of the Italian Government.

[2] On leave of absence from the Gorgas Memorial Laboratory, Panama.

[3] "Ente Regionale per la Lotta Anti-Anofelica in Sardegna" was the designation of the organization which undertook the eradication of *Anopheles l. labranchiae* in Sardinia.

While the Sardinian regional health authorities were continuing to treat all foci of *labranchiae* which could be found, within the limitations of the funds available to them, an agreement was reached to permit the writers to study one focus from which all treatment would be withheld, at least during the 1952 season. The primary objectives of this study were the following:

1. The determination of the rate of increase of a *labranchiae* population in a suitable breeding place in the absence of treatment. One of the curious phenomena which greatly hampered the later work of ERLAAS was that in such *labranchiae* larval breeding places as were found, the larvae were in most cases present in only very small numbers. Factors influencing the finding of larvae under these conditions have in part been brought out by Trapido (1951). The small number of larvae in breeding places in which they formerly abounded indicated that there must be a large element of chance in finding larvae, despite conscientious sampling of all water surfaces. It had been assumed from the general knowledge of the high reproductive potential of mosquitoes and the rapid increase of the population possible in only a few generations, that breeding areas missed on one scouting cycle would be easily found on later scouting. The validity of this assumption was to be tested by carefully following known breeding places throughout the summer, in the absence of treatment.

2. Related to this first objective was the establishment of the rate of dispersal of the species by determining the rapidity with which larvae appeared in breeding places adjacent to the original focus.

3. Adult *labranchiae* had become more difficult to find than larvae as the eradication project had progressed. While this might be due to the persisting effectiveness of residual DDT treatments of all known resting places, it was desirable to find at what larval densities significant numbers of adults again appeared in human habitations and domestic animal shelters.

4. Soon after the ERLAAS plan of attack had been shifted from the treating of all water surfaces (except the water accumulations in tree holes, the breeding place of *Anopheles plumbeus*) to the larviciding of the water surfaces in the areas where *labranchiae* could be found, it became apparent that the other anophelines underwent a shift both in distribution and abundance. The question of how these changes in the balance of the anopheline fauna might affect *labranchiae* through possible competition was also to be considered.

Essential to the investigation of problems such as these was the selection of an area from which treatment could be withheld, but which had a small residual focus of *labranchiae*. Other necessary features of the proposed area of observation were these:

1. That the area be large enough to be representative of the general situation, but small enough to be readily kept under intensive surveillance.

2. That it be enclosed by a natural barrier to limit the spread of *labranchiae* if the population of the mosquito diffused rapidly.

3. That the area be conveniently located for treatment, if the regrowth of the *labranchiae* population was rapid and a malaria transmission hazard developed.

On June 21, 1952, regional scouts found larvae of *labranchiae* along a branch

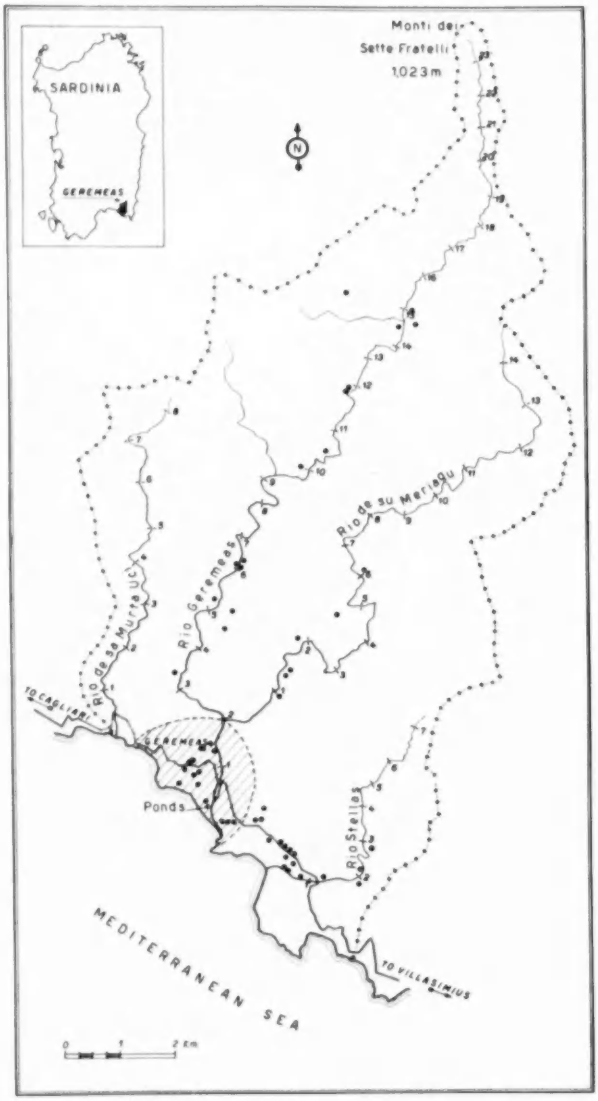

FIG. 1. Sketch map of the Geremeas area, Sardinia. The area of the concentrated studies by the writers is indicated by hatching; the black dots represent houses or shelters in which search for adult mosquitoes was made; the numerals along the rivers indicate distances in kilometers from each river mouth.

of the Rio Geremeas within 750 meters of the point where this river reaches the sea. On June 24, breeding places in the immediate vicinity received a treatment of fuel oil augmented with the spreading agent Triton X-100 (the larvicide did not contain DDT). Further treatment was suspended pending the decision of Professor G. Brotzu, "Assessore all'Igiene, Sanità e Pubblica Istruzione della Regione Autonoma Sarda," as to whether the valley of this river might be used as an observation area during the summer of 1952. Agreement to withhold treatment from the area was reached on July 12, and the active study of the *labranchiae* population was begun as soon as possible thereafter.

The experimental area is shown in the sketch map (see Figure 1). It included the valley of the Rio Geremeas and the tributary Rio Meriagu, together with the adjacent smaller valleys of the Rio sa Murta Uei and the Rio Stellas. It was limited by the ridge of the Serra Marapintau on the west, the Sette Fratelli massif to the north, the ridge of the Serra de Misa on the east, and the sea on the south. The ridges on the sides of the area rise rapidly from the sea to 700 and 800 meters, and in the north to the summit of the Monti dei Sette Fratelli with an elevation of 1,023 meters. The area encompassed was approximately 84 square kilometers.

The region, like practically all of Sardinia, has long ago been deforested. The steeper and less accessible slopes are covered with scrub vegetation, while the more gentle slopes are planted with winter wheat or set out with groves of almond trees. The upper reaches of the valley, where there is older second growth, are now being cut over and the wood burned for charcoal. The only considerable level area is within two kilometers of the sea, and is under cultivation with plantings of winter wheat, vineyards, and almond, citrus and peach trees. The peach orchards are irrigated during the dry summer months, but the irrigation is minimal and water does not stand long enough to support mosquito breeding (see Figure 2).

The bulk of the human population is concentrated in a group of farm buildings about 200 meters from the main river and 1.5 kilometers from the sea (see Figure 3). Here too are concentrated most of the domestic animals, a census of which is given in Table 1. For the rest there are only scattered small shelters, in a few cases of stone but for the most part of brush, in which shepherds and charcoal burners live. The total human population during the summer of the investigation was about 185 persons.

The water in the valley is all contained in the streams and their feeding springs, and several small ponds at the mouth of the main river near the sea. At flood times these ponds become part of the lower course of the stream, but throughout the summer months of the study they remained discrete beside the river bed. With the lack of forest cover on the valley slopes, the winter rains turn the water courses into torrents but during the dry summer months these are reduced to intermittency. The two kilometers of the stream nearest the sea were particularly affected by drying during the summer of the study, but stretches of water remained near the originally-found *labranchiae* foci, and the larger of

FIG. 2. View of the lower portion of the Geremeas valley from a point about four kilometers from the sea, showing the barren open nature of the country and the area near the river mouth under cultivation.

FIG. 3. View of the main group of farm buildings at Geremeas center, including the quarters of farm laborers, a sheep pen, cattle yard, horse stable and pigsty. The one adult *Anopheles l. labranchiae* taken during the study was in the pigsty here.

the ponds, in some cases as much as two meters deep, contained water throughout the summer.

The principal rainfall is during the fall, winter, and early spring months. The summers are hot and dry, with little or no rain. Meteorological data are not available from the Geremeas valley itself, but in Table 2 are given the data on rainfall, temperature and humidity from the Elmas airport, about 35 kilometers northwest of Geremeas. During the period of the study at Geremeas

TABLE 1

Domestic animal census, Geremeas center, Sardinia, August 1952

Cattle	13	Dogs	23
Sheep	370	Cats	22
Donkeys	2	Rabbits	7
Horses	1	Chickens & Chicks	116
Pigs	7	Ducks	26

TABLE 2

Meteorological data from Elmas airport, Sardinia, 1952

	MEAN TEMPERATURE (DEGREES CENTIGRADE)	MEAN RELATIVE HUMIDITY (%)	PRECIPITATION (MILLIMETERS)	DAYS OF RAIN
January	8.1	77	41.2	15
February	8.1	74	23.6	9
March	12.5	75	9.6	4
April	13.2	71	27.6	5
May	17.4	71	9.4	5
June	23.3	68	0.0	0
July	25.6	61	12.4	2
August	25.6	69	0.6	2
September	21.9	69	50.2	8
October	18.3	75	17.4	9
November	13.1	73	41.2	14
December	10.8	82	54.8	13
Total precipitation and rainy days			288.0	86

there was no significant precipitation except a heavy rainfall from September 14 to 17.

While the knowledge of the biology and habitats of the anophelines of Sardinia has been reviewed by Aitken (in Logan *et al.*, 1953) it will be necessary here briefly to describe the main features of the biology of the anophelines present in the valley.

At Geremeas we found *Anopheles hispaniola* to be the anopheline most intimately associated with *labranchiae*. The larvae are characteristic of clear sun-lit water with some freshening by surface movement or by seepage, and are most often associated with growths of the alga, *Spirogyra*. Larvae were not found in water rich in organic material from the decay of aquatic vegetation. The ERLAAS

experience was that the species built up slowly during the summer, becoming most abundant in the last half of the summer and early fall. Unlike *labranchiae*, the adults are not found in houses or domestic animal shelters even in the presence of heavy larval concentrations in nearby breeding places. The adults are thus not subject to the attrition produced on anopheline species that rest in structures treated with DDT or other residual toxicants. The species has been taken in Sardinia from sea level to at least 800 meters, but was not commonly found before 1949.

Anopheles claviger is also a species found in clear fresh water, but is usually in shaded situations. It is also encountered under sun-lit conditions, but usually where the water is cool (ordinarily less than 20°C). Most collections of larvae in Sardinia were made in springs and the rivulets from springs. The species is most abundant in spring and fall, and exhibits a decline in numbers during the hot summer months. Adults are occasionally taken in man-made structures, but the bulk of those found by ERLAAS were resting in natural grottoes or in vegetation. It has been taken as high as 1,300 meters in Sardinia.

Anopheles marteri is a species of the cool shaded waters of mountain streams. It was most commonly associated with *claviger* and only very rarely with *labranchiae*. The few adults taken by ERLAAS were in grottoes or similar natural shelters along water courses.

Quite rare in the Geremeas experimental area during 1952 was *Anopheles algeriensis*. Larvae are usually in still water which is partially shaded. It has been collected in brackish water. While not many adults were taken by ERLAAS, a few were found in man-made shelters.

At the beginning of the eradication effort in 1947, *Anopheles l. labranchiae* was the dominant, most widespread and most generalized anopheline in Sardinia. While it was only rarely found in brackish water, it was widely distributed in fresh-water situations up to 1,000 meters. In this the species differed in its larval habitats from the situations described on the Italian mainland where it is considered a coastal breeder, often in brackish water. Hackett (1949) has remarked on the fresh-water distribution of this mosquito in Corsica, Sardinia, Sicily and the Barbary Coast of North Africa and attributed it to the lack of fresh-water competitors. In Sardinia *labranchiae* was most often taken in sun-lit water, usually with some surface vegetation. The water might be clear, or could be rich in organic material, quiet as in ponds or swamps, or slowly moving as at the margins of streams. At first adults were common in pigsties, stables and houses, but these were all subject to residual treatments with DDT and later Octa-chlor, and adults became exceedingly rare in such situations. In addition to man-made structures they were taken in much lesser numbers in grottoes.

During the anopheline survey of Sardinia in 1946 *labranchiae* was found to be common in the Geremeas valley. Collections of larvae were obtained at a number of places sampled along the course of the stream, and while the detailed records are not available, we know it occurred up to an elevation of several hundred meters and a dozen or more kilometers from the sea. The last *labranchiae* in the valley, prior to 1952, were found in 1949. None were found in the intensive

ERLAAS search during 1950, or the less concentrated scouting conducted by the Regional Government in 1951. The nearest positives during 1950 and 1951 were along the east coast in the vicinity of, and to the south of, San Priamo, an area separated from the Geremeas valley by several high, barren and dry mountain ridges. The year 1952 was thus the third one during which no larviciding was done in the valley. The last DDT residual spraying of houses in the valley by ERLAAS was in September 1949. Although no *labranchiae* had been found here, as part of a general precautionary program the provincial medical authorities had also sprayed houses with DDT on June 5, 1951.

In 1952 the Geremeas valley was under routine observation by scouts of the Regional Government during the months prior to its designation as an experimental area for our use in July. The search was primarily directed at the finding of larvae. Scouting started during the week beginning April 28. Only *claviger* was found consistently during this and subsequent weeks, but in addition there were a few collections of *marteri* in the Rio Bacu Mandara, a tributary of the Geremeas, and in mid-June some *algeriensis* were found in the ponds at the river mouth and in a few water holes in the river bed upstream. As a result of the discovery of *labranchiae* in the lower Rio Geremeas on June 21, the adjacent Rio sa Murta Uci was scouted the following week, but only *marteri* and *hispaniola* were found, and these in the lower part of the stream. At the time *labranchiae* was found on June 21, there was, interestingly enough, little else in the Rio Geremeas, possibly because the river was still running too fast as a result of the spring rains, and there had not yet been enough development of *Spirogyra* to provide stream margin shelter for larvae. It is noteworthy that up to this time in the Rio Geremeas and the tributary Rio Meriagu, *hispaniola*, which was later the dominant species, had not been taken at all. During the critical week when *labranchiae* was first found, the collections consisted of 15 larvae of *labranchiae*, 19 of *algeriensis* and 69 of *claviger*. No adult anophelines were taken during this period.

LARVAL STUDIES

Methods

In order to locate anopheline findings as closely as possible, the streams were marked off in the field into tracts 250 meters long and were given serial numbers. The number of tracts in each river was as follows:

Rio Geremeas	96 (24.0 km.)
Rio Meriagu	56 (14.0 km.)
Rio sa Murta Uci	33 (8.25 km.)
Rio Stellas	30 (7.5 km.)

The first scouts were employed on July 17, preliminary scouting began on July 18, and by the week of July 21 ten scouts were employed. It was subsequently found that this number of men was not sufficient for intensive coverage of the entire area and two additional scouts were added on July 31. The men, working in pairs, were assigned one kilometer of stream to scout each day.

These men had all had previous experience in hunting anopheline larvae and quickly became familiar with the terrain. The crews were rotated so that each pair of men had a different stretch of stream to cover each week. The attempt was made to search the most important parts of the main streams (the Rio Geremeas and Rio Meriagu) each week, and the streams with less suitable *labranchiae* habitats (the Rio sa Murta Uci and Rio Stellas) on alternate weeks. The field work was completed on September 26.

To follow the picture of *labranchiae* distribution and abundance in even greater detail in the lower part of the Geremeas valley where the species was first found, the portion of the Rio Geremeas within 1.5 kilometers of the sea was further subdivided into 50-meter tracts, and all small ponds and pools outside the stream course itself were numbered. Two scouts were assigned exclusively to this area and spent a full week covering it.

TABLE 3

Numbers of anopheline larvae collected by scouts at Geremeas, Sardinia (July–September, 1952)

Anopheles	RIO GEREMEAS	RIO MERIAGU	RIO SA MURTA UCI	RIO STELLAS	TOTAL
labranchiae	242	1	0	0	243
hispaniola	3,917	1,224	281	3	5,425
claviger	376	292	8	71	747
marteri	29	1	25	535	590
algeriensis	10	0	0	0	10
Total	4,574	1,518	314	609	7,015

Apart from the searching necessarily delegated to the scouts, the writers, together with their associate Dr. Sergio Bettini of the Istituto Superiore di Sanità in Rome, undertook to follow certain of the water surfaces in the immediate vicinity of the original positives.

Results of routine scouting

The story of what happened to *labranchiae* is a very simple one, and rather different from what might have been expected in this area in which we knew it had formerly been the dominant species. There was no rapid build-up of the population in the original breeding places, and only a negligible and transitory appearance of larvae in adjacent appropriate breeding places.

The species remained confined to the first five tracts of the Rio Geremeas (within 1.25 kilometers of the sea) throughout the summer, with only two exceptions. On July 10, a scout of the Regional Government, later employed as our chief scout, found four 4th instar larvae in a portion of the Rio Geremeas, subsequently numbered by us as tract 24–25, six kilometers from the mouth of the river at an elevation of 75 meters. An intensive search of this tract by us the following week failed to produce additional larvae, and routine searches by scouts during ten subsequent weeks were also negative for *labranchiae*. The only

other appearance of *labranchiae* outside the small concentration near the river mouth, was a single 3rd instar larva taken by the scouts on September 8 in tract 33–34 of the Rio Meriagu, 10.5 kilometers from the sea at an elevation of 475 meters. The heavy rains of September 14 to 17 flushed out this river so that few anophelines of any species were taken during the following two weeks before the project was terminated, and *labranchiae* was not again encountered away from the mouth of the river.

Of particular interest is the general distribution and large numbers of larvae of *Anopheles hispaniola* taken in the Rio Geremeas and the Rio Meriagu. This species was not found in the anopheline survey of Sardinia by Aitken in 1946, and it was not until late 1949 or 1950 that it was first found in the Geremeas area.

The fundamentally altered relationship among the anophelines is nicely demonstrated by the fact that during the week of the first discovery of *labranchiae* in June, when 15 larvae of this species were taken, no larvae of *hispaniola* were found; yet in the subsequent collections made from July through September, only 243 additional larvae of *labranchiae* were found, while 5,425 larvae of *hispaniola* were taken (see Table 3). Thus during a period sufficient to permit the development of the *hispaniola* population from a level so low that no larvae were found by routine search, up to the point where it was the dominant species, *labranchiae* was barely able to maintain itself.

Results of intensive pond studies

Near the mouth of the Rio Geremeas, within a few hundred meters of the sea, are several depressions, either at the edge of the stream bed which was dry during the summer, or within a few meters of it. The larger of these depressions contained water throughout the summer and a rich growth of aquatic vegetation (*Typha angustifolia*, *Juncus litoralis*, *Scirpus nigrescens*, *Phragmites communis*, *Iris pseudocornus*, *Potomogeton natans*, *Carex* sp., and *Lemna* sp.). The organic richness of the water in these ponds, produced by the decay of the associated vegetation, would we supposed favor the breeding of *labranchiae* over *hispaniola* since the latter species is usually found in clear water with some freshening by circulation. These ponds were all in the immediate vicinity of the originally discovered *labranchiae* foci or were themselves positive at the beginning of the study. We thought that they would be ideal breeding places for *labranchiae* and arranged to follow the growth of the population in them by personal examination each week throughout the summer. Three of the ponds were selected for this study (Nos. 2, 7 and 12). In these ponds the entire water surface intersected by aquatic vegetation was swept by using a large oval net (65 x 30 cm.), and the concentration of surface debris then minutely examined in white enamel basins or tubs (see Figure 4). All anopheline larvae found were microscopically identified in the field and returned alive to the ponds from which they had been taken. The numbers of anopheline larvae taken each week are recorded in Table 4. The interesting result of this study is that only *labranchiae* was found, these breeding places not being invaded by either *hispaniola* or *algeriensis* (the latter

FIG. 4. Concentrated search for anopheline larvae at one of the ponds near the mouth of the Rio Geremeas. The entire intersection surface of the pond was swept with the large net and the accumulated debris transferred to pans where larvae were sought by visual inspection.

FIG. 5. The portion of the Rio Geremeas where studies of the relations between *Anopheles l. labranchiae* and *A. hispaniola* were carried on, both along the stream margin and in the artificial pool in the foreground.

was present in very small numbers close by, near the mouth of the Rio Gere-neas). But, despite the absence of any other anopheline larvae which might have created a situation of competition, the *labranchiae* population did not build up, and in fact no larvae at all were found the last two weeks of September, following the rains of mid-September. The hot, dry weather of August may have resulted in a short life span of adults which would have been unfavorable to the

TABLE 4

Numbers of Anopheles l. labranchiae *larvae in natural ponds at Geremeas, 1952*

	POND NO. 2	POND NO. 7	POND NO. 12	TOTAL
24 July	10	—	—	10
8 August	0	1	22	23
16 August	12	9	20	41
22 August	4	5	13	22
30 August	0	2	10	12
4 September	0	10	45	55
10 September	1	5	7	13
19 September	0	0	0	0
26 September	0	0	0	0
Total	27	32	117	176

TABLE 5

Numbers of Anopheles l. labranchiae *and* A. hispaniola *larvae in artificial pool No. 1, Geremeas, 1952 (dug July 30, 1952)*

	NO. DIPS	*labranchiae*	*hispaniola*	TOTAL	PER CENT *labranchiae*
7 August	20	0	4	4	0
14 August	15	17	7	24	71
20 August	6	27	73	100	27
27 August	5	9	41	50	18
4 September	10	0	14	14	0
10 September	10	0	1	1	0
19 September	20	0	17	17	0
26 September	20	0	66	66	0
Total	106	53	223	276	

rapid growth of larval populations, but there was also no build-up during the cooler weather of September.

Results of intensive artificial pool studies

To provide conditions intermediate between the rich organic waters of the natural ponds and the fresh flowing water of the stream, we dug two pools, two meters in diameter and 20 centimeters deep, about a meter's distance from the river edge, where there would be some subsurface freshening of the water

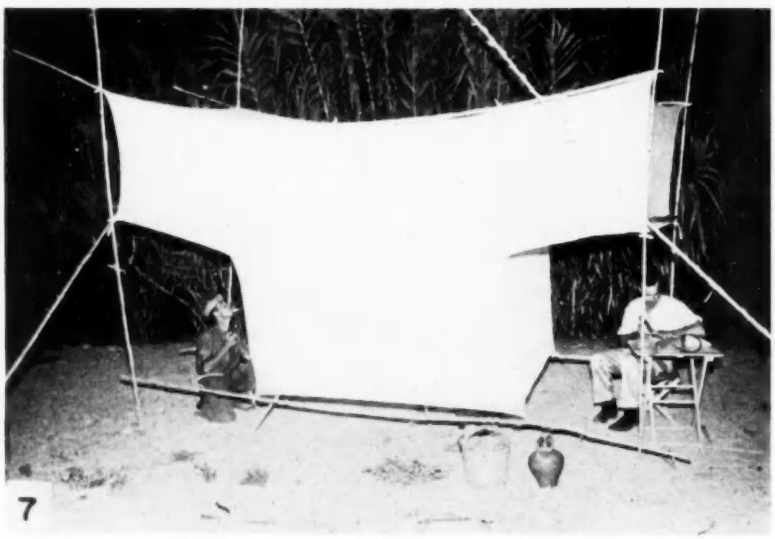

FIG. 6. The white stakes along the margin of the stream mark the four-meter stretches where the balance between larvae of *Anopheles l. labranchiae* and *A. hispaniola* was studied each week. As much as three hours was necessary for the examination of each four-meter stretch. In this breeding place, formerly the habitat of *Anopheles l. labranchiae*, *A. hispaniola* predominated throughout the summer.

FIG. 7. The Shannon light and net trap set up beside a breeding place in the stream at night. Mosquitoes attracted by the light inside the central "box" of the trap were collected by hand. The only anophelines taken by this means were *A. hispaniola*.

through the sandy soil. One of these, which had been dug on July 30, was examined by us each week from August 7 to September 26 (see Figure 5). The pool was seeded with algae and a few aquatic plants from the adjacent stream. Collections were made with conventional dippers and larvae returned alive after identification. The results of the weekly sampling of this pool are given in Table 5. *Anopheles hispaniola* invaded the pool at once, but on the second examination, on August 14, *labranchiae* was dominant, 71 per cent of the larvae being of this species. After this, however, the balance shifted in favor of *hispaniola*, with *labranchiae* disappearing altogether at the last, just as it did in the natural ponds.

TABLE 6

Numbers of Anopheles l. labranchiae *and* A. hispaniola *larvae along stream margin, Geremeas, 1952*

	NO. DIPS	*labranchiae*	*hispaniola*	TOTAL	PER CENT *labranchiae*
25 July	353	16	42	58	28
26 July	239	1	82	83	1
2 August	194	2	52	54	4
7 August	164	3	125	128	2
14 August	131	1	34	35	3
Total	1,081	23	335	358	

Results of intensive stream margin studies

We also attempted a concentrated study of the progress of the balance between *labranchiae* and *hispaniola* in the stream habitat formerly occupied by *labranchiae*. Several tracts of four meters of river margin were examined by us each week until the drying of the lower portion of the Rio Geremeas made the tracts unsuited for anopheline breeding in the latter part of August (see Figure 6). The results of this study are given in Table 6. It will be seen that at all times *hispaniola* was the dominant species.

ADULT STUDIES

In addition to the men employed as larval scouts, two men were engaged to search for adult mosquitoes in human habitations and domestic animal shelters. On the sketch map (see Figure 1) are indicated by black circles practically all man-made shelters in the study area. The types of shelters and the number of times they were examined are shown in Table 7. The net result of this intensive search for adult anophelines was the finding of one adult female *claviger* in a stable at Casa Oghittu, Sa Corti, on August 21, and a single *labranchiae* female in the pigsty at Geremeas center on September 3. Following the finding of this one *labranchiae*, the pigsty was searched daily from Monday through Friday of each week, but no additional specimens were taken.

Samples of wall scrapings were taken at Geremeas center on August 25 for

determination of the amount of DDT residue from the spraying of 1951. Through the courtesy of Professor M. Alessandrini and Dr. V. Amormino, DDT determinations were made at the Istituto Superiore di Sanità, Rome; the results are given

TABLE 7

Inspections for adult anophelines in the Geremeas valley during the 10-week period July 20–September 27, 1952

SHELTER TYPE	NUMBER OF ROOMS		ROOM INSPECTIONS		AVERAGE NUMBER OF INSPECTIONS	
	Geremeas center	Other areas	Geremeas center	Other areas	Geremeas center	Other areas
House	42	61	233	286	5.5	4.7
Stable	8	38	67	199	8.4	5.2
Pigsty	14	11	128	83	9.1	7.5
Hen house	8	6	64	27	8.0	4.5
Rabbit hutch	3	2	22	8	7.3	4.0
Shed	5	16	31	100	6.2	6.2
Shepherd's hut	6	35	33	182	5.5	5.2
Grotto	0	1	0	6	0	6.0
Bridge	0	10	0	37	0	3.7
Total	86	180	578	928	6.7	5.2
Grand Total	266		1,526		5.7	

Note: One *labranchiae* in pigsty on September 3; one *claviger* in stable on August 21.

TABLE 8

DDT determination of wall samples from Geremeas center
Samples collected August 25, 1952[1]

NO. OF SAMPLE	DESCRIPTION OF SAMPLE	WEIGHT OF MATERIAL (GRAMS)	UNALTERED DDT PER SQUARE METER (GRAMS)
1	Whitewashed wall, surface material	0.05	0.10
2	The same, but deeper scraping	0.6	0.32
3	The same, another place	0.08	0.12
4	The same, but deeper scraping	1.00	0.80
5	Wooden beam of pigsty, extracted with acetone on cotton	—	0.40
6	The same, another location	—	0.6
7	Stone wall of pigsty, extraction with acetone on cotton	—	trace
8	The same, another location	—	trace

[1] Analyses performed at the Istituto Superiore di Sanità, Rome.

in Table 8. It is difficult to say how effective these residual deposits ranging from a trace to 0.6 gram per square meter would be in killing adult *labranchiae* under field conditions. But if these mosquitoes were visiting the main group of farm buildings at Geremeas in even modest numbers, more than the one specimen should have been taken in the repeated daytime and nighttime searches.

We also attempted to find adult *labranchiae* in places other than conventional shelters. Adults were sought in tall grass and sedges beside the ponds in which larvae were present, by placing a mosquito net over an area of about 2.5 square meters and blowing smoke into the vegetation with a bee smoker. This procedure was performed 79 times between the end of July and mid-September. While a number of culicine mosquitoes were found by this means, no anophelines were taken.

Anophelines were also sought in rabbit burrows by covering the entrances with netting and spraying in acetone to stir up any mosquitoes inside. About 75 burrows were thus treated during August and early September, but no mosquitoes were found.

In an attempt to find *labranchiae* at night when the adults would be active, we set up a Shannon net and light trap beside a portion of the Rio Geremeas where larval breeding was going on (see Figure 7). The results were as follows:

July 30	8:20 to 10:20 P.M.	2 *hispaniola*
August 18	8:00 to 10:00 P.M.	6 *hispaniola*
August 25	8:00 to 10:00 P.M.	negative
August 27	7:30 to 9:30 P.M.	negative

Two inspections were made at night in the principal pigsty at Geremeas center, but only culicine mosquitoes were found.

MALARIA SURVEY

Two blood surveys of the population of the valley were completed during the period of the study. While 133 thick smears were examined on the first survey and 91 on the second, the actual number of persons from whom blood was obtained on the two surveys was 185. Their age distribution was as follows: (classification after Russell, West and Manwell, 1946):

0–1 year, infants	5
2–4 years, pre-school children	9
5–9 years, school children	21
10–14 years, adolescents	23
15 or more, adults	127
Total	185

One positive slide with a very few parasites of *Plasmodium vivax* was obtained on the first survey from a 56-year-old male without clinical symptoms. The man concerned, a shepherd, lived in the upper valley of the Rio Stellas, an area where no *labranchiae* were found. It was concluded that this was an old infection.

DISCUSSION

It is apparent from the study of this one summer at Geremeas that in the absence of any antimosquito measures, residual populations of *labranchiae* do not necessarily build up rapidly, nor do they necessarily diffuse widely.

This observation might be qualified by noting that Geremeas is a confined valley and the sequence of events might have been different in an open-plain

situation. The failure of the *labranchiae* population to grow or diffuse significantly during the three summer months after its discovery in an area to which it was well suited by the evidence of its prior endemicity remains, however, a most surprising result. If we assume that this residual population of *labranchiae* has the same genetic composition and thereby the same behavior pattern and biotic potential as the population endemic here before the eradication effort, there are three factors which might have mitigated against a normal build-up of the population.

1. The *labranchiae* adult females would have sought their blood meals primarily in human and domestic animal shelters, and the still-persisting residue of DDT on the surfaces of these structures would have produced a mortality great enough so that barely enough females survived to maintain the population. While this possibility cannot be entirely discounted we have no real evidence that this happened, for if *labranchiae* visited the main group of farm buildings in even modest numbers, more than one specimen should have been taken in the repeated daytime and nighttime searches.

2. The *labranchiae* positive was not found at Geremeas until June 21, and if we assume it was the result of a recent introduction, the species may not have become well established until the onset of the hot, dry summer weather, which would have limited the adult life span, thus reducing the number of egg deposits.

3. A third possibility is that there is a critical population density below which there are so few adults in an area that swarming of males and consequent fertilization of females is only partially successful. While the larval population of *hispaniola* was at least as low as that of *labranchiae* at the beginning of the summer, the overwintering adult population of *hispaniola*, which was not detected since the species is not domestic, was probably substantial. The breeding stock of adult *labranchiae*, on the other hand, must have been very small since the species had not been found in the area in the preceding two years.

There remains yet another kind of explanation of what may have happened at Geremeas and in Sardinia, namely that there has been some fundamental alteration in the normal biology and behavior of *labranchiae*, or some change in the balance between it and the other anophelines. The sequence of events at Geremeas points very strongly to the conclusion that if one species of anopheline is eradicated, or nearly eradicated, the vacuum created by its absence may not be permanent, and the lacuna may be filled by another species.

At Geremeas there can be no doubt that *hispaniola* has successfully invaded and occupied what was formerly a *labranchiae* habitat. Whether or not this change in the balance between the two species is a permanent or a temporary phenomenon could only be established by leaving this valley untreated for a period of years, until any DDT residues on structures were reduced to certain insignificance, and *labranchiae* had had several full seasons to express, if it could, the vitality and aggressiveness which formerly characterized it in this area. This will not be possible since the public health authorities considered the malaria hazard too great, and the valley was again put under treatment following the completion of the 1952 observations.

That changes in the hydrology, through drainage and diking which affect salinity and other elements in the larval environment, may modify the balance between members of the *Anopheles maculipennis* group, and in consequence the malaria picture, has long been appreciated and eloquently expressed by Hackett (1937) and others. With the rapid development of a differently oriented attack on anophelines and malaria through the use of residual insecticides, the time has now come to consider whether this chemical attack may not also be so selective that it too would result in an altered balance. In the present case, the residual population of what we call *labranchiae* at Geremeas and in Sardinia may now have as its normal biology and mean behavior pattern a way of life formerly followed by only an insignificant, and thereby overlooked, fraction of the entire population. Specifically, the present *labranchiae* population may not regularly invade human habitations, and the adult habit of selection of oviposition site and source of blood meals, the larval requirements for development and the ability to evade predators may all be altered. If the control of these key functions in the life of the mosquito have a heritable basis, as is likely, then the present residual population of *labranchiae* may lack the genetic materials necessary to express the diversity of habits which resulted in its formerly being the dominant anopheline.

The selective action of residual spraying of human and domestic animal shelters in killing that part of the adult anopheline population which habitually rests in these structures is quite apparent. This phase of the eradication program would have affected the great bulk of the *labranchiae* population, only a small fraction of the *claviger* population, and *hispaniola* not at all. There is a less general appreciation of the fact that even uniform larviciding of all water surfaces would have produced a selective effect on the different anophelines, because both of extrinsic differences in the optimum environment of each species, and intrinsic differences in the habits of the various larvae in the same breeding place. The larvae of *claviger* and *hispaniola* are in situations where there is water movement, or at least some freshening of the water, while those of *labranchiae* are more likely to be in still water. Larvicides would have been more effective, as a result of a longer contact period, in the latter situation than in the former. Even within the confines of a particular breeding place, the differences in reaction of the larvae to disturbance of the water demonstrated by Trapido (1951) would result in a differential exposure, with *labranchiae* more affected than *claviger* or *hispaniola*. Thus it was shown by Trapido (1951) that one minute after disturbance of the water surface, only two per cent of *labranchiae* larvae remained submerged, while 36 per cent of the *hispaniola* were still down. In a test conducted during the present study one *hispaniola* larva survived after being submerged for 75 minutes. Thus even in the situation where larvae of both species were in the same breeding place, *labranchiae* might well be more drastically affected by larvicide treatment.

We must recognize that the behavior of a particular mosquito species or race is not rigid, but capable of a "spectrum" of variation, and that this variation is genetically controlled. The selective action of toxicants on both adults and larvae would result in a residual population which differed in its mean

behavior pattern not necessarily through the addition of new elements by mutation, but with the original genetic material present in different proportions. If the selection were sufficiently rigorous and long enough sustained there would result the total elimination of the portion of the behavior pattern most susceptible to chemical attack. In this situation the lifting of the attack would result in the mosquito no longer being able to re-establish itself in its former way of life, since it would lack the genetic potential to do so. Were the chemical attack only incompletely selective and not sustained for long enough, there would be a gradual reversion to the original situation. In this second case, the factor of the increase and competition from other species would retard the rate at which the reversion would take place. The one-season study at Geremeas was carried on long enough to appreciate the possibility that one or another of these situations may exist, but not long enough to obtain certain proof.

The important implication to be derived from this interpretation of the results is that *labranchiae* at Geremeas, and perhaps elsewhere in Sardinia, has become so modified in its behavior that it may no longer be an effective malaria vector.

SUMMARY

In the absence of any treatment in the Geremeas valley of Sardinia there was no build-up of the population of *Anopheles l. labranchiae* either as larvae in the vicinity of the original focus, or as adults in human or domestic animal shelters. There was no significant diffusion of *labranchiae* from the immediate area of the original positive to other suitable breeding places in the valley. The invasion of former *labranchiae* breeding places by *Anopheles hispaniola* and the replacement of the former by the latter was demonstrated.

The factors which may have contributed to the replacement of *labranchiae* by *hispaniola* are discussed, and it is suggested that the present residual population of *labranchiae* may differ in essential behavior characteristics which would limit its effectiveness as a malaria vector, but the study was not conducted over a long enough period to establish this with certainty.

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SEXUAL ISOLATION IN SOME NORTH AMERICAN ANOPHELES MOSQUITOES[1]

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Morphologically, *Anopheles quadrimaculatus*, *A. freeborni* and *A. aztecus* are very similar to one another, but we now possess sufficient information concerning their habits and certain physiological characteristics to know that they are true species. Although we can be sure of their specific status, these insects are so closely related to one another that it is of interest to compare them biologically, for by such means we may arrive at a better understanding of the mechanisms of speciation among the mosquitoes, and of the manner in which the populations have become adapted to their environments. The purpose of the work with which this paper is concerned was to determine whether the above three species of *Anopheles* are sexually isolated from one another.

The experiments were designed to show whether the males and females of one species would mate more readily with one another than with individuals of another species. Even though a preference for homologous mating might be evident, a high degree of heterologous mating would suggest that selective mating would not operate as an isolating mechanism.

MATERIALS AND METHODS

Colonies of *A. quadrimaculatus*, *A. freeborni* and *A. aztecus* have been maintained in our insectary for several years and were the source of the mosquitoes used in these experiments. Pupae taken from the colony pans were examined under a low-power microscope and segregated according to sex. Adults emerged from these pupae into glass lantern jars; thus there was no contact between the adult males and females prior to the time they were placed in the breeding cages.

The breeding cages were constructed of wire screening, 1 x 1 x 1 foot in size. The adults were one to three days old at the time they were placed in the breeding cages and in most of the experiments they were kept in the cages for three days. In a few cases, particularly when the more active *A. freeborni* males were involved, the breeding period was reduced to two days, and occasionally it was necessary to dissect the females on the fourth day. The adults were fed diluted syrup, and a high humidity was maintained by means of a cylinder of rolled paper towels placed inside the cage in a jar of water and by wet toweling placed over the top of the cage. Each part of an experiment involved the use of two or three breeding cages. These cages containing the adults were placed side by side on a shelf in an air-conditioned insectary and kept under conditions that we have found to be optimum for the maintenance of our stock colonies.

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Because of the similarity in appearance of the adult females, whenever two species of females were mixed in one cage, they were marked prior to being placed in the cage with bronze or aluminum dust. To avoid the possibility that the dust might affect mating activity, in the replicates of the experiments the dusts were alternated between the species. Also, in one experiment, the possible effect of the dusts was studied directly.

EXPERIMENTAL

Experiment 1. The object of this experiment was to determine whether the marking dusts would affect mating activity. Twenty-five males of *A. quadrimaculatus* and 50 females of *A. quadrimaculatus*, *freeborni* or *aztecus* were placed in each of three cages. Half of the females in a cage had been marked with aluminum dust and the other half with bronze dust. This procedure was repeated with males of *A. freeborni* and *aztecus*. The numbers of females from each cage

TABLE 1

Effect of marking dusts on mating activity

MALES	FEMALES; PER CENT POSITIVE					
	quadrimaculatus		freeborni		aztecus	
	B*	A†	B	A	B	A
quadrimaculatus	66	60	10	17	36	44
freeborni	8	12	91	88	70	82
aztecus	8	8	4	26	61	53

* B = marked with bronze dust.
† A = marked with aluminum dust.

that were dissected after a three-day breeding period ranged from 37 to 49. The results are given in Table 1.

Despite the fact that this experiment was performed only once and that a difference may be observed between the bronze and aluminum-dusted *freeborni* females fertilized by *aztecus* males, the results of this experiment, combined with the alternation of dusts in the selection experiments, permit the conclusion that the type of dust may be disregarded as a source of experimental error.

Experiment 2. The object of this experiment was to determine whether the males would mate selectively with females of their own species if given a choice between these and females of another species.

The *A. quadrimaculatus* × *freeborni* combination was set up as follows: 25 male *quadrimaculatus* were placed in a cage with 25 female *quadrimaculatus* and 25 female *freeborni*. At the same time 25 male *freeborni* were placed in another cage with 25 females of each of the two species. Experiments with *A. quadrimaculatus* × *aztecus* and *A. aztecus* × *freeborni* were set up in the same manner. The 1:2 sex ratio was the most satisfactory in that it resulted in the nearest to the ideal of a 50 per cent fertilization of the females. With fewer males, the rate

of fertilization would be too low; with too many males, evidence of selection would be obscured through too great male activity.

The *A. quadrimaculatus* × *freeborni* experiment was performed 5 times and the results are given in Table 2. The *quadrimaculatus* males exhibited a marked preference for mating with *quadrimaculatus* females and fertilized only a few of the *freeborni* females. Greater numbers of both species of females were fertilized by the more active *freeborni* males, but these males also fertilized a significantly higher proportion of their own females.

TABLE 2

A. quadrimaculatus × *A. freeborni*

MALES	FERTILIZATION OF FEMALES*				DIFFERENCE	p†
	Q		F			
	No. dissected	% +	No. dissected	% +		
Q	104	67.3	98	9.2	58.1	<.001
F	98	39.8	96	81.3	41.5	<.001

* Summary of 5 trials.

† Determined from $\sigma_D\% = \sqrt{pq\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}$.

TABLE 3

A. quadrimaculatus × *A. aztecus*

MALES	FERTILIZATION OF FEMALES*				DIFFERENCE	p
	Q		A			
	No. dissected	% +	No. dissected	% +		
Q	87	52.6	64	14.1	38.5	<.001
A	88	2.3	77	51.9	49.6	<.001

* Summary of 4 trials.

The results of the *A. quadrimaculatus* × *aztecus* experiment, which was performed 4 times, are summarized in Table 3. This table shows that *quadrimaculatus* females were seldom fertilized by the *aztecus* males and that although a higher proportion of the *aztecus* females were fertilized by *quadrimaculatus* males, this was significantly lower than the number of *quadrimaculatus* females fertilized by their own males.

The *A. aztecus* × *freeborni* crossing was tested eight times. The results are given in Table 4 and it will be noted that the *aztecus* males fertilized a significantly lower proportion of the *freeborni* females than of their own females. On the other hand, the *freeborni* males fertilized 64.4 per cent of the female *aztecus* and 57.0 per cent of their own females. Apparently the *aztecus* females were more active sexually than were those of *freeborni* and *quadrimaculatus*.

Experiment 3. The object of this experiment was to compare the rates of fertilization of the females of all three species by males of one species, when no choice was possible. Twenty-five males of one species were placed in each of three cages, and to each of these cages were added 50 females of one of the three species. Thus in one cage only homologous mating was possible, while in the other cages the males could mate only with females of one of the other two species. These three cages were set up at the same time with mosquitoes that were of the same age. Each combination was repeated once.

TABLE 4

A. aztecus × *A. freeborni*

MALES	FERTILIZATION OF FEMALES*				Difference	p
	A		F			
	No. dissected	% +	No. dissected	% +		
A	144	59.0	170	10.6	48.4	<.001
F	160	64.4	172	57.0	7.4	—

* Summary of 8 trials.

TABLE 5

Males of all species × *females of all species**

MALES	FERTILIZATION OF FEMALES								p
	Q			F			A		
	No. dissected	% +	p†	No. dissected	% +	p	No. dissected	% +	
Q	96	69.8	—	89	13.5	<.001	80	36.3	<.001
F	82	28.0	<.001	96	68.8	—	86	69.8	—
A	93	6.4	<.001	89	12.4	<.001	84	59.5	—

* Summary of two trials.

† p shows significance of comparisons between the homologous and the two heterologous matings for each species of male.

The results, summarized in Table 5, show that even when the males have contact only with the females of another species, in most cases there still exists a distinct barrier to cross mating. The results are very much like those obtained in the selection experiments, although a few more heterologous matings may take place when no choice is possible. *A. quadrimaculatus* males fertilized more *aztecus* than *freeborni* females. The *aztecus* males fertilized very few *quadrimaculatus* females and a slightly larger number of *freeborni* females. The more active *freeborni* males fertilized 28 per cent of the *quadrimaculatus* females in the nonselection experiments and 39.8 per cent in the presence of their own females. Essentially the same number of *aztecus* females were fertilized by the

freeborni males as were *freeborni* females in both types of experiment; again one may see a greater sexual activity in *aztecus* females.

DISCUSSION

It is evident that a definite barrier to cross mating exists between *A. quadrimaculatus* and the other two species, whereas such a barrier is not so much in evidence between *A. freeborni* and *A. aztecus*. The cross mating experiments as well as the results obtained by Barr (1952a, b) in his temperature and cross-breeding experiments support the conclusions derived from taxonomic evidence that *A. freeborni* and *aztecus* are more closely related to one another than they are to *A. quadrimaculatus*. It is not wise to predict the mating behavior of these mosquitoes under natural conditions from the results obtained in our one-cubic-foot breeding cages; however, one may at least suspect that should *A. quadrimaculatus* coexist in an area with either of the other two species, sexual isolation would operate as an effective barrier. Despite the facility with which *A. aztecus* females are fertilized by *freeborni* males, the fact that comparatively few *freeborni* females were fertilized by *aztecus* males suggests that at least a partial barrier would tend to keep these forms apart should they coexist in one locality. This mating barrier, combined with the lowered vitality of hybrids as demonstrated by Barr (1952b) permits the conclusion that *A. freeborni* and *aztecus* may be regarded as species rather than as subspecies.

Not much is known concerning the mechanisms of species formation among the Culicidae. If one examines a map of the distribution of the members of the *maculipennis* complex in the holarctic region, the impression is gained that the primary barrier may have been geographic, leading to the formation of a typical *rassenkreis*. It is possible that among certain groups, such as the plant-breeding sabethines, adaptation to restricted ecologic niches may have served to isolate populations. However, as Bates (1947) points out, ecological adaptations are manifest most clearly in the aquatic stages and we have no evidence that they would create a barrier to intermingling of the aerial adults. But should adults which had developed in a certain type of larval habitat mate more frequently with one another than with adults originating in another habitat; or should one group of adults become sexually active under one set of environmental conditions while another group is stimulated by slightly different light intensities, temperature and the like, then mating barriers might lead to sexual isolation and eventual species formation.

The cross mating experiments show that mating barriers, varying in degree, do exist between at least three species of the *maculipennis* complex. We have no basis for deciding that these mating preferences arose before or after geographic separation of these species, but the fact that there is only a partial barrier between *A. freeborni* and *aztecus* and that even the more distantly related *quadrimaculatus* females may be fertilized quite readily by *freeborni* males, even after a long period of geographic isolation of these populations, seems to me to indicate that with these species the primary isolating mechanism was not sexual

isolation. However, sexual isolation, having developed to the extent that these experiments suggest is now in operation, would contribute towards maintaining the specific identity of the present-day populations.

SUMMARY AND CONCLUSIONS

Cross mating experiments were designed to show whether sexual isolation exists between *Anopheles quadrimaculatus*, *A. freeborni* and *A. aztecus*.

It was found that a definite barrier to cross mating exists between males of *A. quadrimaculatus* and females of *A. freeborni* and *A. aztecus*, between males of *A. aztecus* and females of *A. quadrimaculatus* and *A. freeborni*, and between *A. freeborni* males and *A. quadrimaculatus* females. However, *A. aztecus* females were fertilized as readily by *A. freeborni* males as were *A. freeborni* females.

These results support conclusions drawn from other studies that *A. aztecus* and *A. freeborni* are much more closely related to one another than they are to *A. quadrimaculatus*. Sexual isolation could be a secondary result of long geographic separation. There is only a partial barrier between the *A. freeborni* and *A. aztecus* and although this might contribute towards a segregation of the two populations, the readiness with which *A. aztecus* females will mate with males of *A. freeborni*, even after a long period of geographic isolation, would lead one to suspect that in this case the primary dividing mechanism was not sexual isolation. This isolation would aid, however, in keeping the present-day populations distinct should their ranges overlap.

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NOTES ON IRANIAN MOSQUITOES

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Between August 5 and November 27, 1949, the writer studied various aspects of malaria control in Iran. During that period his services were loaned by the U. S. Public Health Service to the Imperial Iranian Government and he worked under the auspices of the Seven Year Plan Organization. The purpose of this article is to put on record some of the entomological data obtained during that experience.

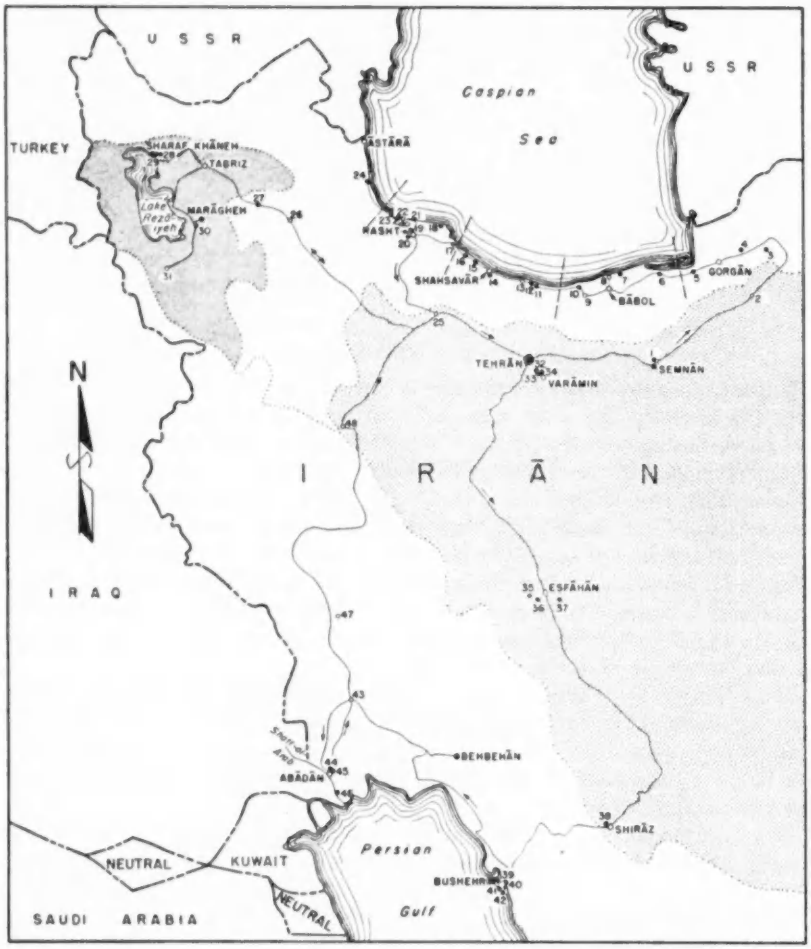
AREAS VISITED

Traveling mostly by automobile, the writer made two extended trips to different parts of central and western Iran. (See map and accompanying list of localities where mosquitoes were collected.) On the first trip, which lasted from August 11 to September 12, the writer traveled east and northeast across the Central Plateau, then crossed the eastern end of the Elburz Mountains and descended to the Caspian Littoral. Working westward along the shore of the Caspian Sea, he spent from three to six nights at four successive bases in the provinces of Gorgan, Mazandaran, Tonekabon, and Guilan. In 1949, this warm and normally humid region was said to be unusually dry, though in accordance with the precipitation gradient which increases toward the west, heavy rain was encountered in Tavalesh on the excursion to Hashtpar.

After leaving the Caspian Basin by the valley of the Safidrud, the writer traveled northwest into the high uplands of Azerbaijan. Here, where bases were established at Sharaf Khaneh on Lake Reza'iyeh at an elevation of about 4,000 feet (1200 m.) and later at Maragheh, the weather in early September was still dry and comfortably warm.

From September 13 to October 24, the writer was principally engaged in making a field comparison of water-wettable and technical DDT near Varamin on the Central Plateau. Certain data from this study are included in this article.

On the second trip, to central and southwestern Iran, the weather at Esfahan (October 25–30) was found to have turned cool. No immature anophelines could be found and none of the captured females deposited any eggs. Since Shiraz, visited October 30–November 1, had also had cold weather, the writer proceeded to Bushehr on the Persian Gulf. After several days at this seaport, the coast was followed to Hendijan, and after a side trip to Behbehan and a stopover at Ahvaz, a last base was established at Abadan in mid-November. Though the climate on the Central Plateau had become quite cool by the end of October, along the Persian Gulf the writer again enjoyed comfortable temperatures quite unlike those of the summer months in that region. Moreover, the dry season did not end before his departure for Tehran. In evaluating the writer's observations, it is important to note that all localities were visited either under dry conditions or after the principal peak of mosquito breeding.



CENTRAL AND WESTERN IRAN

DOTS INDICATE LOCALITIES OF MOSQUITO COLLECTIONS
CIRCLES INDICATE OTHER TOWNS AND CITIES

LEGEND

- ROUTES FOLLOWED BY SURVEY PARTIES
- NATIONAL BOUNDARIES
- PRINCIPAL DIVIDES
- DRAINAGE BASIN OF LAKE REZĀ'IYEH
- DRAINAGE BASIN OF CENTRAL PLATEAU

PSA - PHS - CDC

ATLANTA, GA. JULY 1951

METHODS USED

Adult mosquitoes were usually hunted with a flashlight in homes and animal shelters. They were generally captured with a single-tube aspirator, then blown into paper drinking cups which were covered with fine netting held in place by an elastic band. These containers were used instead of killing bottles in order to obtain eggs from female *Anopheles* of the *maculipennis* group. A hole having the diameter of the aspirator tube was cut in the side of each cup and plugged with cotton. This made it possible to introduce and remove specimens without taking off the netting. To induce egg-laying, water was poured into the cup through the netting and the cup was set aside overnight. Water was never added until the specimens had been brought to the temporary laboratory, and was drained from the cups whenever it was necessary to move headquarters.

The ideal method of making quantitative identifications in the *maculipennis* group is to confine a single female in each cup, but of course all individuals and

Explanation of Map

The dashed lines crossing the southern shore of the Caspian Sea represent the approximate boundaries of the following provinces (east to west): Gorgan, Mazandaran, Tonekabon, Guilan, and Tavalesh (which includes the town of Astara).

List of localities where mosquitoes were collected and of other places along survey routes

		DATES OF COLLECTIONS			DATES OF COLLECTIONS
	Semnan	Aug. 11	27	Gorgabad	Aug. 31
1	Dargazin	11		Sharaf Khaneh	Sept. 2, 3, 5
2	Shahrud		28	Dizagmehr	4
3	Ramian	14	29	Ghanchi	4
4	Aliabad	14		Maragheh	7
5	Gaz	13	30	Khusheh Mehr	8
6	Behshahr	15	31	Mahabad	
7	Roshandan	18	32	Fardis	14–Oct. 9
8	Babolsar		33	Mafiabad	14–Oct. 10
9	Amol		34	Salehabad	14–Oct. 13
10	Mahmud Abad	19	35	Kahruyeh	Oct. 26
11	Kheyrud (river)	19	36	Baghabrisham	28
12	Noshahr	19	37	Dashti	27
13	Chalus		38	Deh Korreh	31
14	Mazardasht	20, 21		Bushehr	Nov. 3, 5, 7
15	Klayebon	21	39	Zolmabad	6
16	Ramsar		40	Alchangi	3
17	Rudsar	23	41	Saratol	4
18	Hassan Kiadeh	29	42	Halileh	7, 8
19	Zarjub	24		Behbehan	10
20	Chomar Saray	24	43	Ahvaz	
21	Ghazian		44	Meherzi	13, 14
22	Koliver	28	45	Fayazi	13
23	Sangachin	28	46	Manyuhi	14
24	Hashtpar	27	47	Dezful	
25	Qazvin		48	Hamadan	
26	Mianeh				

all species cannot be assumed to be equally likely to lay eggs under such conditions or at a given season. Since there was a limited supply of paper cups on the first trip and since the work was done under haphazard conditions, as many as 27 females were sometimes crowded into one container. Under these circumstances, it was possible only to list the different species which oviposited, except that when the females were transferred to another cup and provided with more water, any additional eggs of the same species found on the following day were assumed to have been laid by a different female, and were counted as a separate deposition of eggs.

The aquatic habitats of mosquitoes were studied less systematically than the adult resting places because the main objective in examining breeding places was to find additional species which might occur in a given locality. Except in very shallow water where the mosquitoes could be collected with a medicine dropper, specimens were scooped up in the customary enameled dipper. Besides taking quick dips to catch larvae and pupae before they could escape from the upper layers of the water, "slow dips" were used to collect specimens at or near the surface. Anopheline eggs caught in this way were lifted out of the water on small pieces of filter paper, and were usually preserved in small dry tubes on filter paper moistened with diluted formalin.

Most of the anopheline eggs were identified at the temporary headquarters with a dissecting microscope (prism binocular), but examination of numerous specimens was kindly made by Dr. Augusto Corradetti of the Istituto di Sanità when the writer stopped in Rome on his return trip to the United States. A few larvae were studied during the surveys, and of those brought to the United States some were identified by the writer and others, mounted on slides by Mrs. Margaret Cole of the Communicable Disease Center, were named by Dr. Alan Stone at the U. S. National Museum. Most of the adult anophelines were identified in Iran, but most of the adult culicines, both reared and collected as adults, were carefully identified by Dr. Stone.

The writer wishes to acknowledge with gratitude the special help given by Mrs. Cole, Dr. Corradetti and Dr. Stone, as well as the various persons who assisted him in the field, particularly Mr. Manuchehr Kazemi, Dr. Chamseddine Mofidi, and Mr. Hushang Rafatjah.

NOTES ON THE SEPARATE SPECIES

Because of the confusion resulting from changes in the boundaries of provinces and states (*ostan*), the writer's locality records are cited by the name of the community, which is referred in turn to that of the nearest large town or city (see map and Table 1). When the collection date is not given, it can be found in the list of localities accompanying the map.

Tribe Culicini

1. *Aedes (Stegomyia) aegypti* (Linnaeus)

This house mosquito was reared from collections made in a concrete cistern at Bushehr (Nov. 5 and 7) and in water jugs at Halileh (Nov. 8).

TABLE 1

Adult mosquitoes collected in man-made shelters

LOCALITY	GENERAL LOCALITY	MONTH	CULICINI (ALL SPECIES)		ANOPHELES MACULIPENNIS GROUP		*A. hyrcanus*		*A. superpictus*		*A. spp.* (OTHER SPECIES)	
			M	F	M	F	M	F	M	F	M	F
Aliabad	Gorgan	Aug.	0	2	5	24	1	4	1	0	0	1[1]
Gaz	Gorgan		0	4	2	6	0	0	0	0	0	0
Behshahr	Babol		11	27	1	5	0	0	0	0	0	0
Roshandan	Babol		0	2	2	8	0	0	0	0	0	0
Mazardasht*	Shahsavar		0	3	21	119	0	3	0	0	0	0
Klayebon*	Shahsavar		0	1	25	114	0	0	0	0	0	0
Zarjub	Rasht		1	1	0	1	0	0	0	0	0	0
Hassan Kiadeh	Rasht		2	5	4	50	0	0	0	0	0	0
Koliver	Rasht		1	1	0	0	0	0	0	0	0	0
Sangachin	Rasht		0	1	0	1	0	1	0	0	0	0
Hashtpar	Astara		0	2	6	111	0	18	0	0	0	0
Sharaf Khaneh	Sharaf Khaneh	Sept.	0	2	3	46	0	0	2	186	0	0
Ghanchi	Sharaf Khaneh		0	6	7	19	0	0	6	63	0	0
Dizagmehr	Sharaf Khaneh		0	1	0	6	0	0	1	62	0	0
Maragheh	Maragheh		0	2	1	52	0	0	3	86	0	0
Khusheh Mehr	Maragheh		0	1	3	46	0	0	2	48	0	0
Fardis†	Varamin	Sept.–Oct.	0	0	3	26	0	0	50	801	0	0
Mafiabad†	Varamin		0	0	0	7	0	0	88	880	0	0
Salehabad†	Varamin		0	0	5	36	0	0	125	944	0	0
Kahruyeh	Esfahan	Oct.	0	0	1	125	0	0	0	2	0	0
Dashti	Esfahan		0	0	0	26	0	0	0	0	0	0
Baghabrisham	Esfahan		0	0	0	1	0	0	0	0	0	0
Deh Korreh	Shiraz		0	0	0	76	0	0	0	8	0	0
Bushehr*	Bushehr	Nov.	12	5	0	0	0	0	0	0	1[2]	2[2]
Alchangi*	Bushehr		7	2	0	0	0	0	0	0	0	0
Saratol	Bushehr		0	0	0	0	0	0	0	0	0	1[3]
Halileh	Bushehr		2	1	0	0	0	0	0	0	0	0
Behbehan	Behbehan		3	8	0	0	0	0	0	3	0	1[3]
Meherzi	Abadan		0	0	0	0	0	0	0	0	1[4]	0
Fayazi	Abadan		0	0	0	0	0	0	0	0	0	1[1], 2[4]
Manyuhi	Abadan		19	17	0	0	0	0	0	0	3[4]	0

* Numbers do not represent total of all specimens collected.

† Collections made prior to the spraying in Fardis and Mafiabad, and ending the same week in Salehabad.

[1] *A. pulcherrimus* [2] *A. culicifacies* [3] *A. dthali* [4] *A. stephensi*

In the first of these habitats it occurred with *Anopheles culicifacies*. Its permanent range probably is confined to southern Iran.

2. *Aedes* (*Ochlerotatus*) *caspius* (Pallas)

Three females were collected at Bushehr on November 6 and 7.

3. *Culex hortensis* Ficalbi

One male and two females were reared from collections made at Maragheh in Azerbaijan. The aquatic habitats may be described as follows: puddle in small irrigation ditch beside road; small, springlike pool with little vegetation in pebbly bank of river; and shallow pools with mats of algae in pebbly bed of river exposed to the sun. Other mosquitoes found associated with *C. hortensis* were *Anopheles maculipennis* and *Anopheles superpictus*, *Culex theileri* and *Culiseta longiareolata*.

4. *Culex mimeticus* Noe

Three males and two females of this *Culex*, whose wing markings suggest those of *Anopheles*, were reared from specimens collected on August 19 in a shallow pebbly pool of the Kheyrud, a river about seven kilometers east of Noshahr, a village east of Chalus.

5. *Culex pipiens* Linnaeus

This cosmopolitan *Culex* was found in all parts of Iran where the writer did much collecting except on the Central Plateau. Its absence in his material from that region is probably due mainly to the nature and small number of aquatic habitats examined near Semnan and Varamin, and to the lateness of the season when he was at Esfahan and Shiraz.

Identified adults from man-made shelters were taken in the following localities: Roshandan, Zarjub, Koliver, Sangachin, Hashtpar, Sharaf Khaneh (Sept. 3), Ghanchi, Maragheh, Khusheh Mehr, Bushehr (Nov. 3, 5, 7), Alchangi and Behbehan.

Larvae were found in a small, shallow, shaded stream at Maragheh, and a male was reared from an open well about 3 m. deep at Alchangi.

Other mosquitoes associated with *C. pipiens* in the same aquatic habitats were *Anopheles claviger* and *Culex quinquefasciatus*.

6. *Culex quinquefasciatus* Say

This species, usually called *fatigans* Wiedemann in the Old World, in Iran as in the United States, is of more southern distribution than its close relative *pipiens*. The specimens of both species from southern Iran have been very carefully studied by Dr. Alan Stone of the U. S. National Museum, who dissected and examined the terminalia of many of the males.

Adults of *C. quinquefasciatus* were collected in open wells at Alchangi and Halileh (Nov. 7), and in buildings at Behbehan and Manyuhi.

Three larvae and a reared male were obtained from a large open cistern at Behbehan and a female was reared from a collection made in two open wells at Alchangi.

Associated with *C. pipiens* in the same habitat at Alchangi, *C. quinquefasciatus* was not necessarily reared from the same well. *C. theileri*, however, occurred in the same cistern as *C. quinquefasciatus* in Behbehan.

7. *Culex theileri* Theobald

This *Culex* was common in Azerbaijan, and was found also at Semnan and Behbehan. It was not reared from any locality near the Caspian Sea, nor was it identified in the adult collections from that region. A very few

adults were collected in man-made shelters at Sharaf Khaneh (Sept. 2), Ghanchi, Dizagmehr and Behbehan.

Three larvae were taken in a large open cistern at Behbehan and numerous adults were reared from the following aquatic habitats:—Behbehan: open cistern (in addition to the one mentioned above); Semnan: irrigation reservoir with emergent grass; Sharaf Khaneh: small spring in ditch (Sept. 2); wide irrigation ditch (Sept. 3); watering pool near shore of lake (Sept. 5); water in pasture (Sept. 5); Maragheh: puddle in small irrigation ditch beside road; small, shallow stream with algae and emergent grass in river bed; Khusheh Mehr: small stream with much floating and emergent vegetation.

The following mosquitoes occurred with *C. theileri* in its aquatic environments: *A. maculipennis* and *A. superpictus*, *C. hortensis* and *C. pipiens*, and *Uranotaenia unguiculata*.

8. *Culex tritaeniorhynchus* Giles

With the exception of a single male taken at Behbehan in southern Iran on November 10, this species was found only in the Caspian Littoral where it was one of the two identified species of Culicini from man-made shelters (Aliabad, Klayebon, Koliver). On August 19, larvae were found in a rice field between Babol and Amol and adults were reared from other rice fields in Gorgan (Ramian and Aliabad). Farther west, the species was reared from the following habitats:—Mahmud Abad: pond with floating vegetation; Klayebon: small swamp; Zarjub: canal with emergent grass; Chomar Saray: small brook below spring with mats of glovelike alga; Hassan Kiadeh: shallow pond.

The two other mosquitoes found in association with immature *C. tritaeniorhynchus* were *Anopheles subalpinus* and *Anopheles hyrcanus*.

9. *Culiseta longiareolata* (Macquart)

This species was found only in aquatic habitats and only in Azerbaijan. Adults were reared from the following localities:—Gorgabad, about 70 km. west of Mianeh; Sharaf Khaneh (Sept. 3): well partly covered with boards, about 2 m. in diameter, with water about 2 m. below surface of ground; Maragheh: small, springlike pool with little vegetation in pebbly bank of river.

In the last of these habitats, *C. longiareolata* occurred with *C. hortensis*.

10. *Uranotaenia unguiculata* Edwards

A single female of this pretty mosquito was reared from a collection made at Sharaf Khaneh on September 5 in a marshy pasture along the shore of Lake Reza'iyeh (Lake Urmia). *A. superpictus* and *C. theileri* were reared from the same material.

Tribe Anophelini

Genus *Anopheles*, Subgenus *Anopheles*

11. *Anopheles claviger* (Meigen)

One female was reared from a collection made at Maragheh on September 7. The habitat was a small, shallow and shaded stream, with much emergent

and partly submerged vegetation including some grass. Larvae of *C. pipiens* were taken in the same collection.

12. *Anopheles hyrcanus* (Pallas) var. *pseudopictus* Grassi

The variety *pseudopictus* can be distinguished from typical *A. hyrcanus* as an adult only. In this stage, *pseudopictus* has the fourth segment of the hind tarsus all white or whitish instead of dark except at the tip. The writer has seen 34 adults of the species *hyrcanus* from the Caspian Region, of which 10 are no longer available for examination and 6 lack both hind legs. The remaining 18 are all clearly of the variety *pseudopictus*. Therefore, in spite of the fact that Pallas described the typical variety from marshes about the Caspian Sea, and in spite of the fact that some of the writer's specimens came from the classical land of Hyrcania, all of the writer's collections (including eggs and larvae) are referred to the variety *pseudopictus*. The typical variety *hyrcanus* may also occur in the Caspian area, or it may be that the variety *pseudopictus* is what Pallas actually described. Other varieties of *A. hyrcanus* include *pictus* Loew, and *mesopotamiae* Christophers, which was described from Khorramshahr. Macan (1950, p. 146) has shown that his material of *A. hyrcanus* from western Iran and Iraq appeared to belong to the typical variety, of which Edwards regards *pictus* as a synonym. The exact status and distribution of all the varieties of *A. hyrcanus* should be made the subject of a thorough study.

Adults of variety *pseudopictus*, found in the rooms of houses as well as in animal shelters, were collected in only four of the villages which the writer studied in the Caspian Region (Aliabad; Mazardasht, Aug. 21; Sangachin; Hashtpar). They represented only 5 per cent of the identified *Anopheles* from the eleven villages in the study. Two of the four communities with variety *pseudopictus* were surrounded by extensive rice fields and at one of these (Mazardasht, Aug. 20) the writer was attacked by this mosquito outdoors in the shade in the early afternoon. Two additional specimens were taken at Ramsar (Aug. 21 and 22) and there were three in a collection from Ghoznavi near Gorgan (Aug. 11).

Eggs, larvae and a reared adult were obtained from the following aquatic habitats:—Noshahr: fairly deep channels in river bed with emergent vegetation and surface debris (one female, reared); Mazardasht (Aug. 20): rice fields (11 eggs, 1 larva); Rudsar: quiet river channel (1 egg); Zarjub: canal with emergent grass along banks (7 eggs, 3 larvae); Chomar Saray: small brook below spring with mats of glovelike alga (2 eggs).

Other mosquitoes found associated with *A. hyrcanus* var. *pseudopictus* in one or more of the above aquatic habitats were: *A. maculipennis* and *A. subalpinus*, and *C. tritaeniorhynchus*.

13. *Anopheles maculipennis* (Meigen)

Records of adults of this species taken in man-built shelters are based entirely on eggs laid in captivity (see Table 2). Eggs (their numbers noted in parentheses) were also collected in the field in the following habitats:—Mazardasht: Aug. 20, rice field (2); Sharaf Khaneh: Sept. 2, small spring in

ditch (20); Sept. 3, shady irrigation reservoir (32); Sept. 3, shady irrigation ditch (6); Sept. 3, wide irrigation ditch with floating plants, debris and algae, also emergent grass and sedges (28); Maragheh: puddle in small irrigation ditch beside road (18); Khusheh Mehr: small stream with much floating and emergent vegetation (12).

TABLE 2

Adult Anopheles *of the* maculipennis *group identified by eggs laid in captivity**

LOCALITY	DATE	NO. OF CUPS	NO. OF SEPARATE DEPOSITIONS OF EGGS			
			maculipennis	*subalpinus*	*melanoon*	*sacharovi*
Caspian Region:						
Aliabad	Aug. 14	7	4	1	0	3
Behshahr	15	3	0	0	0	1
Roshandan	18	4	0	0	0	2
Mazardasht	21	8	14	0	0	0
Klayebon	21	11	18	0	0	0
Zarjub	24	1	0	1	0	0
Hassan Kiadeh	29	11	0	6	1?	5
Hashtpar	27	13	5	4	0	0
		58	41	12	1?	11
Azerbaijan:						
Sharaf Khaneh	Sept. 3	9	10	0	0	2
Ghanchi	4	7	8	0	0	1
Dizagmehr	4	2	3	0	0	2
Maragheh	7	7	9	0	0	0
Khusheh Mehr	8	7	4	0	0	0
		32	34	0	0	5
Central Plateau:						
Fardis	Sept. 14	3	0	0	0	1

* Each cup contained from one up to 27 females of the *maculipennis* group. The collections in five additional cups (four from Mazardasht and one from Klayebon) did not produce any eggs. The adults in these collections were never identified and counted, but it is highly probable that each cup contained females of the *maculipennis* group.

Other species of mosquitoes found in aquatic association with *maculipennis* were *A. hyrcanus*, *Anopheles sacharovi*, and *A. superpictus*; *C. hortensis* and *C. theileri*.

Since the name *maculipennis* was formerly used for all members of the *A. maculipennis* group, it is useless to perpetuate records which are not based either directly on eggs or else very conservative identifications of fresh adults. Zolotarev (1945) made a very thorough investigation of eggs of the *A. maculipennis* group from various localities in Azerbaijan as well as the entire Caspian Region and through the kindness of Dr. Alan Stone of the U. S. National Museum, a translation was furnished the writer. For con-

venient comparison, Zolotarev's records of typical *A. maculipennis* may be condensed as follows.

Steppe region of eastern pre-Caspian lowlands: Gunbad-e-Qabus.

Foothills of the eastern Elburz mountains: Kul-tepe, Khadzhiliar, Shahpasan, Gorgan.

Shahi area: Sari, Shahi.

Babol Valley: Babolsar.

Foothills of Elburz mountains and valley of central range: Noshahr-Chalus, Shahsavar, Ramsar, Polirud; Murzanabad.

Delta of Safidrud: Rudsar, Pahlavi.

Valley of Safidrud and road to Qazvin: Siyahrud, Noglebar, Rustamabad, Kagir bridge, Iuzbashchai.

Southern foothills of Talesh and Bagrov mountains: Gashpar (perhaps equivalent to the writer's Hashtpar), Bakhchisarai, Kala, Astara.

Azerbaijan: Mianeh, Ardabil, Bustalavad, Reza'iyeh, Khoi, Baku.

Macan (1950, pp. 194–195) discusses the distribution of *A. maculipennis* in western Iran in terms of geological formations and their possible effect on larval habitats, but fails to reach any satisfactory conclusion. On the other hand, he finds that the July isotherm of 84° F. follows quite closely the western limit of *A. maculipennis* in the Zagros Mountains.

14. *Anopheles melanoon* subspecies *melanoon* Hackett(?)

Eggs tentatively referred to this subspecies were obtained only once. Four females of the *maculipennis* group which were collected in the stable of the caviar cannery at Hassan Kiadeh on August 29 were placed in a paper drinking cup to which water was added that evening. The next day there were eggs of *A. melanoon* subspecies *subalpinus* in the container, and the cup was drained before the writer's departure for Qazvin. The next evening, in Tabriz, only one female was still alive. Water was again provided, and the following day (Sept. 1), the cup contained a batch of very dark eggs. Dr. Augusto Corradetti agreed with the writer that these eggs appeared to represent the subspecies *melanoon*, but it seems best to consider their identity in doubt until there is further proof that the subspecies occurs in Iran.

15. *Anopheles melanoon* subspecies *subalpinus* Hackett and Lewis

This form was found only in the Caspian Region. The records are based on eggs laid by females which had been caught in man-made shelters (see Table 2), and on eggs collected in the following habitats:—Rudsar: quiet river channel, 5 eggs; Zarjub: canal with emergent grass along banks, 4 eggs; Chomar Saray: small brook below spring with mats of glovelike alga (?), 1 egg (identification doubtful); Hassan Kiadeh: rain barrel, 5 eggs; shallow pond (?), 2 eggs (identification doubtful).

Other mosquitoes found associated with *subalpinus* in its larval habitats were *A. hyrcanus* and *C. tritaeniorhynchus*.

Zolotarev (1945) reports 3 eggs masses of *A. maculipennis* out of 51 from Ardabil as being similar to those of *subalpinus*, and 6 doubtful egg masses of *subalpinus* from Bakhchisarai in the southern foothills of the Talesh and

Bagrov mountains. Otherwise, he found *subalpinus* mostly in the valleys of the Babol and Safidrud, as shown in the following summary:—Shahi area: Sari, Shahi; Babol valley: Babolsar, Mahmud Abad, Suladeh; northern foothills of the Elburz Mountains: Noshahr-Chalus, Shahsavar (?), Polirud; Delta of Safidrud: Rudsar, Shalmaneh, Lahijan, Rasht, Pahlavi, Dinachal.

16. *Anopheles sacharovi* Favr

The writer's records of this species are based entirely on eggs, mostly those laid by captive females (see Table 2). Noteworthy is the occurrence of *A. sacharovi* at Hassan Kiadeh which seems to represent a considerable extension of the known range. At Sharaf Khaneh in Azerbaijan, 6 eggs were collected on September 3 in a shady irrigation reservoir and numerous eggs were found on September 5 in a pasture on the nearby shore of Lake Reza'iyeh where irrigation seepage had formed shallow puddles. That no eggs were discovered in aquatic habitats in the Caspian Region may be partially explained by the writer's less intensive searching east of Noshahr. Zolotarev (1945) says that Beklemishev and Gontaeva (1943) report *A. sacharovi* from the areas of Qazvin and Lake Reza'iyeh, but he found the species only in the provinces of Gorgan and Mazandaran. His records may be summarized as follows: Otoliar, Gunbad-e-Qabus, Gorgan airport, Gorgan, Sari, and Babolsar. Macan (1950, pp. 182–190) notes that in western Iran, except on one of six routes crossing the Zagros Mountains from west to east, *A. sacharovi* "was not found beyond the highest valley below the line along which there is an outcropping of igneous rocks." In general, he found *A. sacharovi* extending further west than *A. maculipennis* and occurring at much lower elevations.

Genus *Anopheles*, Subgenus *Myzomyia*

17. *Anopheles culicifacies* Giles

This Indian species, the only positively identified anopheline found during the writer's surveys in the vicinity of Bushehr in early November, was not collected elsewhere in Iran. Adults were found in a concrete cistern at the Custom House (Gomrok) in Bushehr (Nov. 5 and 7), and in another cistern at Saratol. Numerous adults were reared from collections of larvae and pupae taken in two cisterns at the Custom House in Bushehr (Nov. 5 and 7), and other lots of larvae presumably of this species were taken in two other cisterns in Bushehr (Nov. 3 and 7), one at Saratol, and two at Zolmabad.

18. *Anopheles dthali* Patton

The only mosquito positively identified as *dthali* was an adult female taken in Behbehan on November 10.

19. *Anopheles pulcherrimus* Theobald

The writer collected two females of this species, both in houses: one at Aliabad near Gorgan, and one at Fayazi on Abadan Island.

This is perhaps the species represented by "one damaged specimen of what appeared to be A. stevensi" which was collected by Roberts (1950, p. 7) at Kushkibidak in the Shiraz valley. This possibility is suggested in view of

the known ranges of both species and the fact that both have more or less speckled legs.

20. *Anopheles stephensi* Liston

Six adults of this Indian species were collected on the writer's surveys, all in man-built shelters on Abadan Island: one male at Meherzi (Nov. 13), 2 females at Fayazi and 3 males at Manyuhi. At Meherzi (Nov. 13 and 14), many larvae were collected from two shallow puddles in pits about one meter deep. These puddles, apparently of ground water origin, were completely exposed to the sun.

21. *Anopheles superpictus* Grassi

The writer collected only one adult of this widely distributed species in the Caspian Region (Aliabad), but adults were very abundant in man-built shelters both in Azerbaijan and on the Central Plateau near Varamin. Near Sharaf Khaneh and near Varamin, they far outnumbered individuals of the *maculipennis* group (see Table 1). In the late fall, 2 females were taken at Kahruyeh near Esfahan, 8 females at Deh Korreh near Shiraz, and 3 females at Behbehan.

Immature stages of *A. superpictus* were collected at several localities. The identification of the eggs noted in the following list rests principally on the fact that *A. superpictus* was the only *Myzomyia* found in Azerbaijan:—Dargazin: small muddy area beside shallow stream (2 reared adults); Klayebon: pebbly margin of small river (25 reared adults); Sharaf Khaneh: Sept. 2, small spring in ditch with matted algae and debris including straw (3 eggs and 6 reared adults); Sept. 3, wide irrigation ditch (record based on 2 eggs); Sept. 5, puddles in irrigated pasture near lake (record based on eggs only), marshy pasture near lake (eggs and 2 reared adults); Maragheh: shallow pools with mats of algae in pebbly bed of river, exposed to sun (5 reared adults); pool in small irrigation ditch with some emergent grass (record based on 8 eggs); Khusheh Mehr: small stream with much floating and emergent vegetation (record based on 15 eggs).

Other mosquitoes associated with *A. superpictus* in the larval habitats were: *A. maculipennis* and probably *A. sacharovi*, *C. hortensis* and *C. theileri*, and *U. unguiculata*.

DISCUSSION

In collecting adult mosquitoes, it was an easy matter to sample the populations occurring in the vicinity of man. Most Iranians, especially in the rural areas, live in similar mud-walled houses in close association with their domestic animals. Sometimes the living quarters are in a story above the stables, but generally both are reached from a common courtyard. Moreover, in the arid parts of Iran, the typical village is so compact and the streets and alleys so bare of shade that there are few spaces other than man-made shelters which are suitable for those mosquitoes that rest in dark places during the day.

Throughout the Caspian Region and Azerbaijan, the procedure in inspecting villages was sufficiently uniform to permit some quantitative as well as qualitative comparison of the different areas (see Table 1). When there was not enough time to make a thorough search of all the roofed space used by one family, it

was customary to inspect a minimum of one room and one stable or other animal shelter in each home visited. Though females of the *A. maculipennis* group (and also of *A. superpictus*) were quite generally less common in rooms occupied by man, eggs of *A. sacharovi*, typical *A. maculipennis*, and *A. subalpinus* were laid by specimens caught there as well as in stables. It is well known, of course, that the diurnal resting habitats of *Anopheles* are not necessarily the places where they feed. Actually, in this day of controlling malaria by residual insecticides, the important point to determine is whether the types of man-made shelter which can be readily and safely sprayed are also the usual resting places of the principal vectors.

On various kinds of evidence, the important vectors of human malaria in western Iran, both known and probable, can be limited to the following species: *A. sacharovi*, *A. maculipennis*, *A. superpictus*, *A. stephensi*, and *A. culicifacies*. Since each of these, as shown in Table 1 and confirmed by other observers, has been found resting in man-made shelters, the second question is, to what extent does the adult population of each species rest in buildings. In the dry regions of Azerbaijan and the Central Plateau, the lack of other daytime habitats appears to concentrate *A. superpictus* as well as *A. sacharovi* and *A. maculipennis* in the villages. Probably *A. superpictus* is a partially wild species in the moist Caspian lowlands (where only one specimen was found in a home). In fact, one of the observations on this behavior by Macan (1950, pp. 202–203) was duplicated by the writer at Klayebon where a rocky stream in which the larvae of *A. superpictus* were abundant was within a few yards of houses from which he did not obtain a single adult. Since *A. sacharovi* and *A. maculipennis* are more abundant in the Caspian Region and of definite house-frequenting habits, the control of *A. superpictus* by indoor residuals may never present a real difficulty.

In southwestern Iran, the problems of malaria control are more circumstantial. Fortunately *A. stephensi*, of great importance at Abadan, appears to rest commonly in ordinary buildings, but at Bushehr, adult *A. culicifacies* was taken only in the large covered cisterns where the winter rains are collected and stored for use during the dry season. Since they are dark, cool and not adequately screened, they provide ideal breeding and resting places in which spraying with a toxic insecticide would be most inadvisable. When this minor difficulty has been resolved, perhaps by an engineer, there appears to be no entomological obstacle, other than the behavior of *A. superpictus* in humid areas, to the final control of malaria in western Iran.

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NATURAL INFECTION OF THE TICK, *AMBLYOMMA CAJENNENSE*, WITH *RICKETTSIA RICKETTSII* IN PANAMA

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The occurrence of Rocky Mountain spotted fever on the Isthmus of Panama was established for the first time in 1950 when a highly virulent strain of *Rickettsia rickettsii* was isolated by the author from the blood of a 26 year old Panamanian farmer who died February 8 of that year, shortly after admission to the hospital. The patient evidently had contracted his infection near the town of Ollas Arriba about seven miles north of Capira in the Province of Panama. Since then similarly virulent strains of *R. rickettsii* have been isolated from two additional cases, hospitalized in May 1950 and February 1951, both male, aged 13 and 26 years respectively, who were employed in agricultural work on farms located in the same area as the previous case. The 13-year old boy died. The 26-year old man survived after a severe and protracted illness. His blood showed complement-fixing antibodies against Rocky Mountain spotted fever antigen in high titer during convalescence.

These findings indicated that the vicinity of Ollas Arriba was an endemic focus of Rocky Mountain spotted fever. However, nothing was known of the possible vectors. In order to determine the identity of the vector, we began to collect ticks in the town of Ollas Arriba and neighboring sections, including the towns of Caimito, Caimitillo and Valdesa. The collection was begun in February 1952 near the beginning of the dry season and continued through July of the same year. The cooperation of Drs. G. B. Fairchild and P. Galindo of the staff of the Gorgas Memorial Laboratory was received in planning and conducting the field work. The area was divided into four zones which were swept periodically for ticks by dragging a white flannel cloth over the ground. The ticks which accumulated on the cloth were killed by freezing, brushed off, classified and used for guinea pig inoculations. The drag technique yielded large numbers of larvae and nymphs, but relatively few adults. Later we began to pick ticks from horses which were brought to the Ollas Arriba station from the neighboring areas with a high yield of adults.

No attempt was made to isolate *Rickettsia* by feeding the ticks on experimental animals due to the fear of escapage. Instead groups of these arthropods were ground with physiological salt solution. The resultant suspension was centrifuged lightly and the supernatant solution used to inoculate paired guinea pigs of which one received one cc. of the inoculum by the subcutaneous and the other by the intraperitoneal route. Temperatures were taken once daily for a period of one month, the animals being observed for any abnormality at the same time.

All animals were bled for serological study both prior to inoculation and 30 to 35 days afterwards. The complement-fixation reaction with Rocky Mountain spotted fever antigen (soluble type) purchased from Lederle Laboratories was uniformly employed, the technique used being that of overnight ice-box incuba-

tion as recommended by Cox. It was hoped by this means to detect any inapparent infections such as those described originally by Spencer and Parker (1923) who found that the inoculation of guinea pigs with suspensions of macerated fasting infected ticks may produce an asymptomatic but immunizing form of the disease. Tests with Q and murine typhus fever antigens also were performed, as controls, and in hope of identifying a natural vector of Q fever in this area.

Only three different species of ticks were recovered, *Amblyomma cajennense*, *Amblyomma oblongoguttatum* and *Otocentor nitens*. A total of 2436 adult *A. cajennense*, 194 *O. nitens* and 24 *A. oblongoguttatum* were obtained, also about 8000 nymphal *Amblyomma* and an innumerable quantity of larvae. We are indebted to Dr. G. B. Fairchild for all tick identifications. Adult ticks were classified as to species and nymphs as to genera. No attempt was made to classify larval ticks.

TABLE 1

Data concerning guinea pigs giving positive results after inoculation with suspensions of macerated ticks

NO. OF GUINEA-PIG	INOCULUM	DATE OF INOCULATION	ISOLATION OF *R. rickettsii*	TITER OF COMPLEMENT-FIXING ANTIBODIES AGAINST RMSF ANTIGENS IN SERUM
709 & 710	A. cajennense, 11 adults from horse	March 19	Yes	—
694 & 695	A. cajennense, 25 adults, drag	March 3	No	1-32 & 1-16
728	1000 non-identified drag larvae	March 22	No	1-16
829	A. cajennense, 58 adults, drag	June 10	No	1-8
903	A. cajennense, 23 adults from horse	July 12	No	1-32

RESULTS

A strain of *R. rickettsii* highly virulent for the guinea pig according to the criteria established by Topping (1941) was isolated from a group of 11 adult *A. cajennense* picked on March 19, 1952, from a horse which had been brought from an orange grove located near the town of Caimito to the Ollas Arriba station. Both guinea pigs inoculated with a suspension of these ticks developed fever 5 and 6 days respectively after inoculation. The strain was readily established in passage guinea pigs by serial subinoculations, producing high fever of 3 to 8 days duration after an incubation period of 3 to 4 days, a marked testicular reaction and high mortality (90 per cent). Postmortem examination revealed characteristic gross pathology including enlargement of the spleen, inflammation of the inguinal and axillary lymph nodes and inflammation of the testicles and scrotum accompanied frequently by adhesions between the visceral and parietal lamina of the tunica vaginalis, hemorrhages into the tunica and scrotum and necrosis of the scrotum. Scrapings of the tunica vaginalis revealed small numbers of typical rickettsiae. Cross-immunity studies between this tick strain and a strain of *R. rickettsii* previously isolated by the author from a fatal case of the

disease showed complete reciprocal cross-immunity. Furthermore, 8 guinea-pigs vaccinated with a commercial vaccine all survived a challenge inoculation with the tick strain whereas 8 controls all died.

No symptoms suggestive of Rocky Mountain spotted fever were obtained in any of the other guinea pigs inoculated with tick suspensions. However, 5 animals showed a change in serology from negative prior to inoculation to positive in titers of 1–8 to 1–32 thirty to thirty-five days after inoculation. These positive animals included guinea pigs no. 694 and 695, both injected on March 3 with the same suspension of 25 adult drag *A. cajennense*; no. 728 inoculated on March 22 with a pool of approximately 1000 non-identified drag larvae, no. 829 inoculated on June 10 with a suspension of 58 adult drag *A. cajennense* and no. 903 inoculated on July 12 with a suspension of 23 adult *A. cajennense* picked from a horse. Results of this study are presented in Table 1. The complement-fixation reactions with Q and murine typhus fever antigens gave completely negative results.

DISCUSSION

The isolation of a strain of *Rickettsia rickettsii* from one lot of adult *Amblyomma cajennense*, as well as the demonstration of complement-fixing antibodies against Rocky Mountain spotted fever antigen in the serums of guinea pigs inoculated with other lots of this species, provides evidence that *A. cajennense* is a vector in Panama. This species has been found naturally infected also in Colombia by Patiño-Camargo (1941), in Mexico by Bustamante and Varela (1946) and on various occasions in Brazil according to data summarized by Dias and Martins (1939). A related species, *Amblyomma americanum*, was found spontaneously infected in the United States by Parker, Kohls and Steinhaus (1943). Although *Otocentor nitens* has been shown by Patiño-Camargo (1941) to transmit the infection under experimental conditions, no evidence of natural infection was found in the limited number examined here. Furthermore, the habits of this species render it improbable that it would play an important role as a vector. We have as yet had no opportunity to examine specimens of *Rhipicephalus sanguineus*, a species which was found naturally infected with Rocky Mountain spotted fever in Mexico by Ortiz Mariotte and co-workers (1944). However, as Fairchild (1943) reports that this species is very rarely found on any animal except the dog in Panama, it is of doubtful importance as a possible vector here.

Amblyomma cajennense has all the qualifications of the perfect vector in Panama. It is not only one of the most abundant ixodid species in this area but also exhibits a wide host range and attacks man readily in all stages. Under the circumstances it is difficult to understand why clinical Rocky Mountain spotted fever is not encountered with greater frequency. Evidently further investigatigation of the many epidemiological factors involved is required.

CONCLUSIONS

A strain of *R. rickettsii* highly virulent for the guinea pig was isolated from a lot of 11 adult *Amblyomma cajennense* removed from a horse in an endemic area

of Rocky Mountain spotted fever in Panama. Guinea pigs inoculated with three other lots of this species remained asymptomatic but developed complement-fixing antibodies against Rocky Mountain spotted fever antigens in their serums.

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SCHISTOSOMIASIS AND LIMNOLOGY IN THE AMAZON REGION

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In October, 1949, the Medical Department of the *Plantações Ford de Belterra* (Drs. E. Soubihe Naufal and W. Gillet Machado) discovered the first autochthonous cases of schistosomiasis *mansoni* in Fordlandia. Examination of a part of the population revealed nearly 30 cases of schistosomiasis, among them several children who were born at and had never left Fordlandia. In 1950 Dr. Rainéro Maroja of the Serviço Especial da Saúde Pública (SESP) in a more thorough examination of the population found an even larger number of infected persons (Verbal communication from Dr. R. Maroja, 1951.)

According to communications from the Health Department of the State of Pará and the National Public Health Service to the Medical Department of the Plantações Ford de Belterra, no case of autochthonous schistosomiasis had ever been reported from the Amazonian region prior to 1949; the cases found up to that time were in immigrants from highly infected regions of Northeastern Brazil. It must be kept in mind that there has been an almost constant immigration into the Amazon region from these sections since the second half of the last century, and among these immigrants there have certainly been many schistosomiasis carriers.

Regarding schistosomiasis in the Amazon region, Maria Paumgartten Deane, working at that time for SESP, writes (1947, p. 12): "As for trematode infections, they apparently do not occur in the region. Some American texts state that *Schistosoma mansoni* is endemic in the Amazon Valley, but this is a mistake probably caused by the misinterpretation of data seen in a publication by Davis of the Rockefeller Foundation concerning his findings in the examination of liver sections taken post-mortem by viscerotomy for detection of yellow fever cases. Among 2613 liver sections of people dying in the Amazon region, Davis found six with *S. mansoni* eggs. Such cases are, however, explained by the migrations of people from Northeast Brazil where the disease is endemic. No autochthonous case has ever been found in the Amazon and all those discovered by SESP (three) or private doctors, had contracted their infection in other areas. No intermediary hosts have thus far been encountered in the region, although they have been sought for during the extensive examinations of water collections made for the study of mosquito breeding places."

Also Madureira Pará, who made examinations of viscerotomy material from all of Brazil during the decade 1937–1946 and found a very small percentage of infections with *S. mansoni* in the territories and states of the Brazilian Amazon region (among 26,825 examined livers only 60 infected ones = 0.22 per cent), concludes (1949, p. 527): "Pará is one of the states of the Union where the stream of immigrants from the Northeast is most intense. For this reason the viscerotomy findings regarding the presence of schistosomiasis in most of the

Amazon region should be regarded with reservation, since they may constitute imported cases."

And Jair Nery, 1952, who in that year made a thorough coproscopic examination of the population in some parts of the lower Tapajós River near Fordlandia, from São Luiz to Aveiro, found 10 cases of schistosomiasis among 1687 fecal specimens, but all in immigrants from the Brazilian Northeast and not one autochthonous case.

The problem is, therefore, not how schistosomiasis was introduced into Fordlandia, but rather why this disease is still endemic only in Fordlandia and has not become established in much larger areas.

Hydrobiological and hydrochemical studies in Fordlandia

In September, 1950, I was designated by Dr. Felisberto C. de Camargo, the Director of our Institute, to study the biological and limnological aspects of the endemic schistosomiasis at Fordlandia. Fordlandia, an area of about 3500 hectares, was cleared of the original jungle between 1929 and 1934 for the purpose of planting rubber trees. The plantations are located on hills coming down to the Tapajós River. The small valleys between the hills usually have swift streams or small creeks, sometimes with pools of stagnant water. There exist about half a dozen "acampamentos," which are groups of houses inhabited by plantation workers with 5 to 25 families in each. The main village of Fordlandia is located on the shore of the Tapajós River and, with the "Vila Americana," is supplied with filtered river water. The "acampamentos" get their water from dammed up springs or directly from creeks or from both.

Schistosomiasis had been found on first investigation by the Medical Department of the Plantações Ford de Belterra only in the Acampamento da Pedreira. However, the later examination by Dr. R. Maroja revealed its existence in all of the "acampamentos" with the main focus in the Acampamento da Pedreira. Limnological examinations of the creeks and springs were carried out by the author in 1950. These studies consisted in collecting hydrobiological material in practically all creeks in the inhabited area of Fordlandia for the purpose of finding out which were infested with planorbid snails. At the same time samples of the waters were taken for chemical analyses.

Planorbid snails proved to exist in all but one of the creeks (Igarapé do Aderson) at Fordlandia. In one ditch in the Acampamento da Pedreira, where the greatest number of schistosomiasis cases have been found, planorbid snails occurred by the thousand per square meter of bottom. In the other creeks their number was not determined, but it was certainly much less. The fact that these snails were not found in the Igarapé do Aderson does not mean that they can not or do not exist there since a rapid collection was made in one place only on this creek. The finding of an organism in some biotope is proof of its occurrence, but failure to find it on one examination alone is not proof of its absence. The chemical composition of the water of the Igarapé do Aderson is similar to that in the other creeks and watersnails of the genus *Potamopyrgus* (family: Amni-

colidae) occurred in all the creeks so that there is no visible reason why planorbids could not live there as well.

The watersnails of Fordlandia have been classified by Dr. Fritz Haas, Curator of lower invertebrates of the Chicago Natural History Museum. The commonest

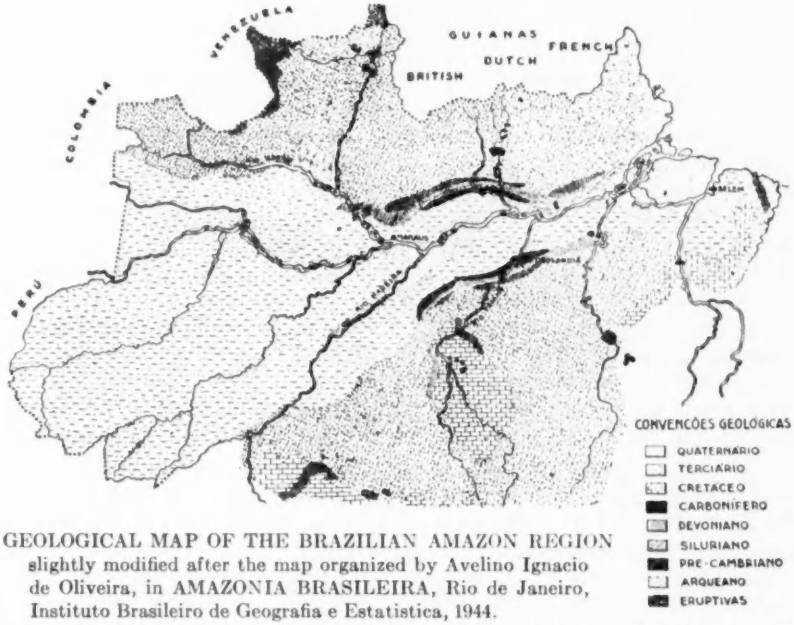
TABLE 1

Chemical properties of waters of the Amazon Region

	FORDLANDIA WATERS EXAMINED IN SEPTEMBER AND OCTOBER, 1950		WATER OF THE RIO TAPAJÓS IN THE DIFFERENT SEASONS	WATERS WITHOUT OCCURRENCE OF WATERSNAILS (FOR THE ONLY EXCEPTIONS CF. P. 702)
	8 Creeks	2 Springs		
Turbidity, colour and transparency in m. (determined by the Secchi-Disk)	crystalline to opalescent	crystalline	green to olive green. 1.80–4.30	crystalline or green to brown. 1.30–3.15
Temperature in °C	24.7–34.3	26.4–26.6	27.7–32.6, and up to ≧ 37 among floating water plants and algae in clear sunlight.	24.4–32.2
pH	6.7–≧7.6	5.4–5.5	6.4–6.65, and up to ≧ 7.6 among floating water plants and algae in clear sunlight.	4.1–5.50
Dissolved O_2 mg/l	6.6–9.0	2.6–3.2	6.8–9.2	1.4–8.0
Free CO_2 mg/l	0.75–6.0	87.0–95.25	0.7–3.5	2.2–91.5
Bicarb.–CO_2 mg/l	19.8–94.6	19.8	2.7–5.2	0–7.0
Humic acids mval/l	0.055–0.135	0.08	0	0–0.1
Hardness in °DH (German Hardness Degrees)	0.85–2.90	1.40–1.50	0.31–0.82	0–0.65
Calculated as $Ca^{\cdot\cdot}$ mg/l	6.1–20.3	10.0–10.7	2.2–5.9	0–4.6
Total Fe ($Fe^{\cdot\cdot} + Fe^{\cdot\cdot\cdot}$) mg/l	0.13–0.40	0.05–0.15	0–0.12	0–0.68
$Al^{\cdot\cdot\cdot}$ mg/l	0	0	0	0–traces
$Mn^{\cdot\cdot}$ mg/l	0	0–0.1	0	0–traces
NH_3 mg/l	0–0.12	0	0.07–0.18	0–0.18
Dissolved SiO_2 mg/l	12–27	19	5.0–6.5	2.5–9
Cl' mg/l	0–5.2	0–0.2	≦0.5	0–3.0
SO_4'' mg/l	0	0	0	0–traces
P_2O_5 mg/l	0–traces, below 0.05	0	0	0
N_2O_5 mg/l	0.25–2.30	1.95–2.00	traces–0.3	0–1.9
$KMnO_4$–consumption mg/l	7.6–44.1	9.8–10.1	12.7–25.1	2.0–141.0

species of the family Planorbidae is *Tropicorbis* (*Obstructio*) *paparyensis* F. Baker, which was found in almost all creeks of Fordlandia (exceptions: Igarapé do Cacaual, Igarapé do Aderson and Igarapé Arraia). This is also the only species of Planorbidae in the ditch of the Acampamento da Pedreira, the main

focus of schistosomiasis in Fordlandia, and must therefore be considered as the main, if not the only vector of the disease. Two other species of Planorbidae, found only in two creeks of Fordlandia, are *Gyraulus* (*Drepanotrema*) *anatinus* Orbigny, in the Igarapé do Cacaual, and *Gyraulus* (*Drepanotrema*) *schubarti* Haas, in the Igarapé Arraia. Since there is no "acampamento" near this creek, only one short collection and no chemical analysis of its water have been made. Therefore, since practically all creeks of Fordlandia are inhabited by planorbid snails, the whole area of Fordlandia may be regarded a zone in imminent danger of the spread of schistosomiasis.



GEOLOGICAL MAP OF THE BRAZILIAN AMAZON REGION
slightly modified after the map organized by Avelino Ignacio de Oliveira, in AMAZONIA BRASILEIRA, Rio de Janeiro, Instituto Brasileiro de Geografia e Estatistica, 1944.

FIG. 1. Geological Map of the Brazilian Amazon Region. (Reproduction of the geological map by Avelino Ignacio de Oliveira, in Amazonia Brasileira; slightly modified.)

Fordlandia waters are characterized by a pH close to neutrality and by a relative abundance of dissolved salts. These peculiarities generally occur in waters of the Carboniferous regions of Lower Amazonia (see geological map, Fig. 1) (cf. Sioli, 1950, and Braun, 1952). Therefore, in chemical properties the waters of Fordlandia (Table 1) may serve at the same time as examples of the waters of all the Carboniferous regions of Lower Amazonia.

The results of the chemical analyses of waters in other parts of Amazonia may throw some light on why schistosomiasis has not yet become established in the whole Amazon region. Therefore, to show the differences, Table 1 contains also the limit values of the chemical properties of such waters where they have been examined, especially of the Tertiary and Devonian parts of lower Amazonia

and the Archean zone of the upper Rio Negro. The differences of the watersnail faunas will be discussed below.

The studies of the writer and of Dr. Rudolf Braun, who collaborated in 1947–48, show fairly clearly in what types of waters planorbid snails usually occur. All species of Planorbidae (classification made by Dr. Fritz Haas, Chicago) found in the Amazon region since 1940 are listed below, together with the pH values of the waters in which they were collected. Since the particular species of Planorbidae which may act as intermediate hosts for *Schistosoma mansoni* are not precisely known, all species of this family are enumerated in the following list.

1. *Gyraulus* (*Drepanotrema*) *anatinus* Orbigny:

In shore-lakes in the *varzea* (flood-plain) zone of white-water and clear-water rivers such as the Amazon and the Rio Madeira, the Rio Juruena and the Rio Tapajós: pH 6.0–6.9. In the Rio Cuparí system (in the Carboniferous zone like Fordlandia, see Sioli, 1949): pH 6.3–7.1. Fordlandia, Igarapé do Cacaual: pH 7.4.

2. *Gyraulus* (*Drepanotrema*) *kermatoides* Orbigny:

In the system of the Rio Cuparí: pH 6.4.

3. *Gyraulus* (*Drepanotrema*) *depressissimus* Moricand:

In the Rio Cuparí: pH 6.6.

4. *Gyraulus* (*Drepanotrema*) *schubarti* Haas:

In shore-lakes of the *varzea* of the Amazon and the Rio Madeira: pH 6.5–6.6. Fordlandia, Igarapé Arraia: pH 6.8.

5. *Tropicorbis* (*Obstructio*) *paparyensis* F. Baker:

In shore-lakes of the flood-plain of the Rio Solimões, Amazon, Rio Madeira, Rio Tapajós, Rio Cuminá: pH 5.70–6.9. In the Rio Cuminá, Rio Tapajós, system of the Rio Cuparí: pH 6.05–7.1. On the shore of the Rio Tapajós, among floating waterplants and algae: pH from 6.4 in the open river to $\geqq 7.6$ among the floating waterplants and algae in clear sunlight. In almost all creeks of Fordlandia: pH 6.7–$\geqq 7.6$.

Because of its abundant occurrence at the main focus of schistosomiasis in Fordlandia (Acampamento da Pedreira) in the absence of other species, this is probably the most important if not only intermediate host of *Sch. mansoni* in Fordlandia.

The main biotope of all planorbid snails in the larger bodies of water are the roots of floating waterplants.

For comparison, examples may be cited of more acid waters which have been thoroughly studied by the author and which have never contained planorbid snails or watersnails of any other families (except for the rare occurrence of four species of the family Ancylidae): creeks and lakes of "*terra firme*" (not subject to periodic flooding) of Tertiary origin in the region of Santarem-Belterra and in the geologically identical terrain west of the lower Rio Tapajós, pH $\leqq 5$; creeks and artificial lakes of the "terra firme" of Tertiary origin at the Colonia Estadual de Tomé-Assú, on the Rio Acará-pequeno, south of Belém-Pará, pH 4.90–5.50; creeks and shore-lakes in the region around the Missão São Francisco

do Cururú, probably of Cretaceous origin, at the Rio Cururú, tributary of the upper Rio Tapajós, pH $\leqq 5$; creeks in the Archean zone of the upper Rio Negro, pH 4.1–5.2.

Also the larger rivers which belong entirely to the Tertiary part of lower Amazonia, as the Rio Acará-pequeno and the Rio Arapiuns, and the upper Rio Negro (Archean zone), are free of watersnails with the exception only of the cited Ancylidae and one species of the family Ampullariidae (with extremely thin shell, not yet all classified but probably all *Ampullarius* (*Ampullarius*) *papyraceus* Spix); pH of these waters was 4.3–5.0.

These observations indicate that the absence of watersnails, including Planorbidae, is typical of all strongly acid creeks, springs and lakes in the Amazonian "terra firme" of Tertiary and Cretaceous origin (cf. Sioli, 1950, p. 279) and in the zone of Archean origin, at least in the special conditions of the upper Rio Negro.

The most important factor determining the occurrence or nonoccurrence of watersnails in the different bodies of water seems to be the pH of the water rather than the poorness in dissolved salts ($Ca^{\cdot\cdot}$). The reason for this supposition is suggested in the column "Rio Tapajós" in Table 1. As the table shows, the Tapajós water is as poor in dissolved salts as the waters in which watersnails do not occur. The only notable difference consists in the higher pH of the water of the Rio Tapajós which may probably be accepted as the expression of a lower content of free, "aggressive" CO_2 in the practically unbuffered waters with their low content or absence of HCO_3.

Inferences as to the danger of establishment of schistosomiasis in different parts of Amazonia

The following inferences can be drawn, from the distribution of planorbid snails, regarding the possible spread of schistosomiasis in Amazonia.

Planorbid snails exist in almost all bodies of nearly neutral water. In the strips of the terrain of Carboniferous origin (see geological map) planorbids occur in many creeks of the "terra firme" which, for the most part, have more or less neutral water. The snails also occur in various bodies of water (e. g., in shore lakes) on the *varzea* shore (flood-plain) of the white-water rivers (Sioli, 1950), and they also live near the banks, especially among the floating shore-vegetation of all the larger white and clear water rivers examined.[1]

[1] It should be explained that in Amazonia one can distinguish three different types of rivers:

"White-water" rivers, with muddy, turbid water as of the Amazon itself or the Rio Madeira which generally have their origin in the Andes; their water is rich in suspended particles and relatively rich in dissolved salts; the pH is very near to the neutral point (Amazon: pH 6.9).

"Clear-water" rivers, which have more or less green, transparent water as, for example, the Rio Tapajós or the Rio Xingú; they come generally from the older, flatter regions of Central Brazil; their water contains little suspended matter and is poor in dissolved salts; the pH is around 6.5.

"Black-water" rivers, which have transparent but dark brown water, as the Rio Negro

Planorbidae have not been found in the acid creeks and springs of the "terra firme" of Tertiary, Cretaceous and Archean origin. As the geological map shows, most of the Amazonian Hylaea is occupied by these formations. Therefore, it may be concluded that there need be no fear of establishment of schistosomiasis in the Tertiary, Cretaceous and Archean "terra firme" regions of Amazonia even though it should be introduced by migrants from the outside.

Unfortunately for any future colonization scheme, these are the regions with the poorest soils in Amazonia; for some time to come they will not give sufficient basis for dense settlement. The best soils are those of the *varzeas* or flood-plains of the white-water rivers such as the Rio Solimões, the Amazon itself, the Rio Madeira, etc. Because of the floating shore-vegetation in these rivers and in their shore lakes, this zone may well be able to spread schistosomiasis when the river and lake banks become densely populated. This stage will not be reached for some time.

Since the population of Amazonia now lives mainly along these river banks, the question arises, why no widespread schistosomiasis has developed here although the disease has certainly been imported often enough throughout the whole area. The following circumstances are of importance in this connection: 1) The Amazonian population does not have the custom of defecating in water as do, for example, the natives of Indonesia. It is therefore difficult for the *Schistosoma* eggs in the feces to reach the water for further development and eventual infection of the relatively rare Planorbidae. 2) The settlers of the Amazonian river banks rarely bathe among the floating vegetation where planorbids occur, and prefer waters where the shore is cleaner. 3) If a *Schistosoma* carrier should defecate in the water near the floating vegetation, the probability of the planorbid snails becoming infected is still low. The volume of the water is so great and the planorbids so infrequent in this biotope that the miracidia will only rarely find a planorbid host.

The best soils for farming on the Amazonian "terra firme" are undoubtedly to be found in some parts of the Carboniferous bands along the Lower Amazon Valley; they are formed in part by weathering of the diabase and may have limestone deposits (Sioli, 1949). Should agricultural activities begin here, and should these regions become densely settled, the danger of schistosomiasis will be great. The creeks—the preferred source of drinking, washing and bathing water—have for the most part almost neutral waters. They are frequently inhabited by planorbid snails, the potential hosts of *Schistosoma mansoni*. The Carboniferous bands of the Lower Amazonia may consequently become danger centers of schistosomiasis in the Amazon region as shown in the case of Fordlandia where this has already happened.

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or the Rio Cururú (tributary to the upper Rio Tapajós); their origin is in terrestrial shapes where the evolution towards the peneplain has made greatest progress and where there are great stretches of flooded jungle (so called "igapó"). They are also poor in suspended particles and extremely poor in dissolved salts; the brown color of their water is due to dissolved organic matter (humus substances) which is extracted from the "igapó"; the pH is very low, from 4.3 (upper Rio Negro) to 5.2 (Rio Cururú).

SUMMARY

The limnological conditions at Fordlandia (State of Pará, Brazil), at present the only known place with endemic schistosomiasis *mansoni* in the entire Amazonian region, are propitious for the development of planorbid snails since the Carboniferous formation is responsible for surface waters of almost neutral reaction, which seems to be a requisite for survival and multiplication of these snails, as shown by surveys of many Amazonian areas where waters are acid and these snails are absent. It is concluded that, with colonization by immigrants from Northeast Brazil and other regions infected with schistosomiasis, the disease may become endemic in the regions with Carboniferous strata north and south of the Amazon, but there is little danger of this in the rest of the great area of the Amazon basin.

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A COMPARISON OF THE DOSAGE-MORTALITY RESPONSES OF SOME STRAINS OF *AUSTRALORBIS GLABRATUS* TO SODIUM PENTACHLOROPHENATE

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The effectiveness of sodium pentachlorophenate (NaPCP) as a molluscicide against *Australorbis glabratus* has recently been demonstrated. Nolan and Berry (1949) concluded that this chemical showed promise as a molluscicide in field trials in Texas against *Tropicorbis obstructus donbilli*, a snail closely related to *A. glabratus*. Preliminary tests in this Laboratory against a strain of *A. glabratus* from Venezuela had suggested the use of this chemical for field studies. The data from these tests were reported later, [Nolan, Bond and Mann (in press) and Berry, Nolan and Oliver Gonzalez (1950)]. In the latter study, NaPCP was found to be effective against *A. glabratus* in field trials in Puerto Rico when applied at 10 p.p.m.

In the laboratory tests mentioned above, concentrations of 3 to 10 p.p.m. killed 100 per cent of the Venezuelan strain in 24 hours. Mortalities at 2 and 1 p.p.m. were 80 per cent and 10 per cent, respectively. In laboratory tests in Brazil, Szumlewicz and Kemp (1951) obtained 100 per cent mortality with "*A. immunis*" at concentrations of 3 to 10 p.p.m., and 98 per cent and 95 per cent at 2 p.p.m. and 1 p.p.m., respectively, in 24 hours. In further tests with "a species of *Australorbis* which is a proved intermediate host of human schistosomiasis," comparable results were obtained. Also in Brazil, Wasicky and Unti (1951) in tests with *A. glabratus* obtained 100 per cent kill at concentrations of 2 to 10 p.p.m., and 80 per cent kill at 1 p.p.m. in 24 hours.

These results pointed to the rather complete effectiveness of NaPCP at concentrations above 2 p.p.m. regardless of the source of the snails, although there was variation in results at lower concentrations. However, in some instances there was apparent uncertainty as to whether or not a snail in question was *A. glabratus*. Furthermore, because the laboratory studies in most instances involved the evaluation of a few to many chemicals, the number of snails used in testing a chemical was varied and sometimes necessarily rather small. The question arose as to whether there are differences in response to NaPCP among different strains of *A. glabratus*, particularly at the lower concentrations. Inasmuch as several such strains have been maintained routinely in this laboratory, it was decided to compare the susceptibility of some of these strains to this chemical.

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MATERIALS AND METHODS

All snails used in these studies belonged to the species, *Australorbis glabratus*. The various strains studied had the following histories: Snails of the Venezuelan, Brazilian, and Puerto Rican strains were descendants of a few snails sent in to the Laboratory from their respective areas 3 or more years ago. The Brazilian mutant strain (Bm) was composed of albino snails with a red appearance and was derived from a single albino snail which was isolated from the above-mentioned Brazilian wild-type pigmented strain (Bwt) shortly after the latter had been established in the Laboratory. Inasmuch as snails of the Venezuelan strain were used by Mrs. M. O. Nolan for molluscicide screening, they had been maintained continuously in large numbers prior to their use in these studies. Snails of the other strains had been maintained in much smaller and more varied numbers, which were increased just prior to and during these studies.

Two methods of exposing the snails to NaPCP were used. As a preliminary procedure, a 5-gallon aquarium was set up with aerated tap water, gravel and aquatic vegetation. Forty to 50 adult snails of approximately the same size and without pitted shells were added. The following morning the snails were recounted, and sufficient technical grade NaPCP was added to result in a calculated concentration of 0.5 p.p.m. The water in the aquarium was aerated and stirred vigorously during application of the chemical. At irregular intervals throughout that day and subsequent days, the aquarium was checked and snails which appeared to be dead were removed and placed into fresh water. They remained in the latter overnight and were checked again the next morning. If a snail recovered, it was returned to its aquarium. While the latter procedure introduced a complicating factor, so few snails recovered it was of little consequence.

This was continued until all the snails in a test had died, or until only a few survivors remained alive over a period of several days. At the outset, and thrice weekly, samples of water were taken from the aquarium for residual NaPCP determinations. The use of this procedure was shortly abandoned in favor of a method which appeared to introduce fewer variables and to provide a more suitable means for detecting and expressing possible differences among the strains.

The other procedure, from which most of the data reported herein were obtained, differed from the above as follows: Twenty snails of an approximately uniform size (14 to 16 mm. in diameter) were placed in a 1-gallon battery jar which was filled to within ¼ inch of the top with a solution of pure NaPCP in aerated tap water. The chemical was prepared from C.P. pentachlorophenol and sodium bicarbonate. The jar was covered with a notched glass plate to prevent the escape of the snails. A pipette, which was connected to an air line, was introduced into the jar through the notch, and air was passed gently through the fluid. In this manner, the latter was kept mixed, and the air at the surface was replaced continuously.

The exposure period used in this series of tests was 24 hours. The concentra-

tion of NaPCP applied was varied in multiples of 0.25 p.p.m. and the range of concentrations used for a strain was arbitrarily chosen as that which would include average mortalities ranging from less than 10 per cent to 90 per cent or more. The temperature of the room in which the tests were conducted was under some regulation and averaged 80° F., varying from 78° to 84° F.

At the end of the 24-hour exposure period, a sample of the fluid in a jar was removed for residual NaPCP determination. The treated snails were then transferred from the jar to a beaker and rinsed with fresh water. The apparently dead snails were separated from those which appeared to be alive and both groups were placed into fresh water, with a piece of lettuce, for another 24-hour period. At the end of the latter, mortality was determined.

Usually, six jars of snails were exposed simultaneously in a single day's test. It was not possible to include all the several strains, nor to run the complete range of concentrations required for a strain in a single test mainly because the supply of snails of the appropriate size varied. However, attempts were made to include at least two different strains in a test. Also, when more than one jar of snails of a given strain was to be exposed, different concentrations of chemical were used rather than replicates of the same concentration. This was done in an attempt to spread the possible variation from one batch to another among all strains and concentrations.

The method used for the determination of the NaPCP concentration of the solutions to which the snails were exposed was a modification of the method described by Haskins (1951). This method is based on the formation of a chloroform-soluble complex between pentachlorophenol and either methylene blue or safranin-O. The spectrophotometric procedure using safranin-O as the complexing agent was found incapable of producing reliable results with solutions containing less than 1 p.p.m. sodium pentachlorophenate. It was found, however, that by using a 20 ml. sample and methylene blue that satisfactory results could be obtained with concentrations as low as 0.1 p.p.m.

The procedure was as follows: For concentrations of 0.1 to 0.5 p.p.m., a 20-ml. sample was taken; 0.75 to 1.0 p.p.m., a 10-ml. sample; and 1.25 to 1.5 p.p.m. a 7-ml. sample. The sample was placed in a 40-ml. centrifuge cone and diluted to 20 ml. if of a lesser volume. Four ml. of methylene blue reagent, prepared as described in the reference above, was added and followed by 5 ml. of chloroform. The tube was stoppered and shaken vigorously for 30 seconds. The layers were allowed to separate. The chloroform extract was then removed with a capillary pipette and transferred to a 1-cm. cuvette. The optical density was read at 655 millimicrons in a Beckman spectrophotometer. For the blank, the chloroform extract from a similarly treated 20-ml. sample of the water used for the preparation of the pentachlorophenate solution was used. The results were read from a density-concentration curve prepared from analysis of pentachlorophenate solutions of known concentration. This curve was a straight line over the range of 0.1 to 0.5 p.p.m. but did not pass through the origin; it crossed the concentration ordinate at approximately 0.02 p.p.m. The density reading at 0.5 p.p.m. averaged 0.450, at 5 p.p.m. averaged 0.215, and at 2.5 p.p.m. averaged 0.108. The analyses were made in triplicate.

RESULTS

Most of the data obtained in the preliminary tests in which aquaria were used were derived from comparisons of the Venezuelan and Bm strains. The supply of suitable snails at the time was greatest for these strains. Furthermore, because of the color difference, it was possible to expose both strains simultaneously in the same aquarium and thus to make a direct comparison.

Determinations of residual NaPCP at various periods after application varied among the tests but indicated that the chemical dissipated rather slowly. From an average initial concentration of 0.51 p.p.m., the average residual at 1 week was 0.41 p.p.m. and at 2 weeks was 0.33 p.p.m.

In Table 1 are presented the mortality results obtained with these two strains at various intervals during the first week of exposure. The figure at each interval is the mean of the mortality percentages obtained in five tests with 19 to 25 snails of each strain in each test. As can be noted, all members of the Bm strain were dead by the third day of exposure (80 hours as the data are tabulated). None of

TABLE 1

Comparative mortalities of Bm vs. Venezuelan strains of Australorbis glabratus *at intervals after application of 0.5 p.p.m. NaPCP to aquarium*

Strain	MEAN PER CENT MORTALITY ± S. E.*				
	8 hours	32 hours	56 hours	80 hours	1 week
Venezuelan	0	<1.0	11.4 ± 6.9	29.0 ± 12.9	66.6 ± 8.3
Bm	0	67.8 ± 9.1	94.2 ± 2.0	100	—

* Derived from the mortality percentages obtained in five tests with 19 to 25 snails of each strain per test.

the snails of this strain which were considered dead and placed into fresh water ever recovered.

In contrast were the results obtained with the Venezuelan strain. While there was variation among tests in the proportion of snails dead at a particular time, the first few days were characterized by relatively little mortality in all tests. By the end of the third day only about one-third of the snails of this strain had died. Some snails survived for as long as 2 to 4 weeks. However, because of a decreasing chemical concentration, it cannot be said that these snails survived the original conditions of exposure for this long. An occasional snail thought to be dead recovered when placed into fresh water. However, all such instances occurred during the later stages of a test.

It was apparent from these preliminary tests that there was a susceptibility difference between the Venezuelan and Bm strains to the lethal action of NaPCP. Further studies with these strains confirmed this difference.

The results obtained with the second series of comparisons are shown in Table 2. In this series, studies were also made with the Bwt strain, and some information was obtained on the Puerto Rican strain. In Table 2, the 24-hour residual NaPCP determinations are presented as means for all the tests at a particular applied concentration. These determinations indicated no dissipation of the

chemical during the exposure period, averaging, in fact, slightly higher than the calculated applied concentrations.

In general, there was a characteristic pattern in the results of exposures in which at least some snails were killed. At the end of the 24-hour exposure period some were obviously dead. These were completely retracted, had a dull opaque appearance, and some had gas bubbles in their tissues suggestive of decomposition. Such snails never recovered when placed into fresh aerated water. The snails which did not appear to be dead at the end of the exposure behaved in several ways when removed to fresh water. Some glided normally around the sides of the container and did not appear to have been affected. Some started hemorrhaging and dropped to the bottom where they remained. Others had the anterior portion of the body extended considerably beyond the edge of the shell and remained in that position. Such snails had a distended and reddened ap-

TABLE 2

A comparison of the 24-hour mortality among strains of Australorbis glabratus *after exposure to sodium pentachlorophenate*

SODIUM PENTACHLOROPHENATE CONCENTRATION—p.p.m.		MORTALITY—MEAN PERCENTAGE ± S.E.			
24-hour residual mean ± S.D.	Applied	Brazilian mutant*	Brazilian wild-type*	Venezuelan*	Puerto Rican†
0.27 ± .01	0.25	0.0	—	—	—
0.50 ± .02	0.50	27.5 ± 5.1	3.3 ± 1.7	3.3 ± 2.1	6.7
0.76 ± .03	0.75	59.5 ± 4.8	33.3 ± 10.3	16.7 ± 3.3	—
1.05 ± .06	1.00	92.5 ± 1.7	94.2 ± 1.6	49.2 ± 7.3	55.0
1.34 ± .03	1.25	—	—	68.4 ± 4.2	—
1.53 ± .15	1.50	—	—	90.8 ± 3.0	97.5

* Six tests, 20 snails per test, at each concentration.

† Only two or three tests at each concentration.

pearance and seemed to be anesthetized in that they exhibited little or no response to touch. Practically all snails in the latter two categories died during the 24-hour period allotted for recovery.

Each mortality figure presented in Table 2 is the mean of the mortalities obtained in six tests (20 snails to a test) at each concentration, with the exception of the Puerto Rican strain. For the latter, sufficient snails were available for only two or three tests at a particular concentration. The difference in susceptibility between the Venezuelan and Bm strains noted in the preliminary tests was again in evidence. For example, only a small percentage of the Bm snails survived a concentration of 1 p.p.m. for 24 hours, whereas about one-half of the Venezuelan snails did so. Approximately the same conclusion is reached when the Bwt strain is compared with the Venezuelan. Apparently the latter strain is somewhat more resistant than the two Brazilian strains.

Of particular interest was the apparent difference between the Bm and Bwt strains. While this was not detectable at 1 p.p.m., at lower concentrations the two strains responded differently to the chemical. Whether or not the difference

was associated with the fact that one strain is pigmented and the other is an albino is not known.

In Figure 1 are presented the probit lines fitted by Karber's method (Cornfield and Mantel, 1950) to the mortality data obtained with the Bm, Bwt, and Venezuelan strains. For these computations it was assumed that all 120 snails exposed to a given concentration were drawn from the same population. It was also assumed that the variation in experimental conditions occasioned by the fact that all 120 were not exposed simultaneously did not significantly influence the mortality results. The standard errors presented in Table 2 indicate that, on the whole, these were not improper assumptions. The probit lines differ from one

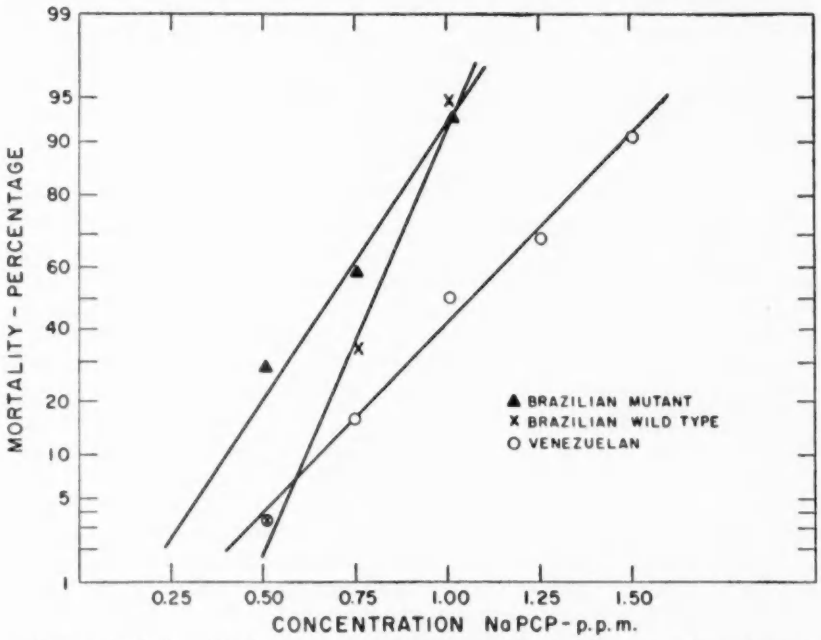

FIG. 1. Probit lines fitted to dosage-mortality data obtained with three strains of *Australorbis glabratus* exposed to sodium pentachlorophenate for 24 hours at 80°F.

TABLE 3

LD_{50} and slope of probit lines fit to mortality data*

STRAIN	LD-50 (p.p.m.)	SLOPE (PROBITS PER 0.25-p.p.m. DOSE UNIT)
Brazilian mutant	0.68 ± .016	1.14 ± .047
Brazilian wild-type	0.80 ± .013	1.79 ± .112
Venezuelan	1.06 ± .020	0.79 ± .029

* Fit by Kärber's method.

another both in displacement toward increased dosage and in slope. A comparison of these characteristics, expressed mathematically, is presented in Table 3. Both the LD_{50}'s and the slopes were found to differ significantly among the three strains. Thus, there is a difference among these strains not only in "average" susceptibility, as measured by the LD_{50}, but also in homogeneity as measured by the slope.

Detailed analysis of the data obtained with the Puerto Rican strain was not performed because of the limited number of tests possible. However, the findings (see Table 2) suggest that this strain is rather similar in response to the Venezuelan strain.

DISCUSSION

It should be borne in mind that the dosage-mortality curve obtained for a strain in these tests may not necessarily be descriptive of the snails, as a group, in the respective area from which the strain originated. These data were obtained with descendants of, and many generations removed from, a rather small sample of a snail population of a particular locality of the area. The Bm strain, in fact, was originally derived from a single snail. Also, as indicated earlier, there were differences in the numbers of snails maintained for the various strains prior to these studies. The Bm, Bwt, and Puerto Rican snails were increased in numbers prior to and during these studies, from a single aquarium or two. Snails of the Venezuelan strain had been maintained continuously in many aquaria. Variation of this kind could account for differences in slope if the latter is due to genetic variation within the strain, and especially if several genetic factors are involved. Furthermore, such variation in population maintenance levels could be expected to occur under natural conditions. Thus, in spite of the difference obtained between the laboratory strains, it is doubtful whether one could come to a fair conclusion regarding the comparative susceptibilities of field Venezuelan and Brazilian *A. glabratus* to NaPCP, at least until samples from several localities of one area had been compared with samples from several localities of the other area.

From the practical point of view, the differences in susceptibility among the strains tested, while significant, were not marked. At a concentration of 2 p.p.m. for 24 hours, it seems likely that few, if any, snails in any of the strains would survive. In field trials with NaPCP, sufficient chemical has usually been applied to result in calculated concentrations of 5 to 10 p.p.m. Assuming that proper distribution of the chemical and adequate contact periods prevailed, such concentrations could be expected to provide uniformly good results as long as the population or strain variation encountered in the field was no greater than that found in these studies. However, the very demonstration of significant strain differences leaves open the possibility that populations with flatter slopes and greater LD_{50}'s may exist or may arise. Such populations, if sufficiently resistant, could pose isolated control problems and could be sources from which even more resistant populations might be selected.

The results obtained in these studies suggest that differences in susceptibility to NaPCP have, at least in part, a genetic basis. However, it is to be expected that other factors associated with the snail, such as age, size and general physiological state at the time of exposure will also determine whether or not a given snail will succumb to a particular set of exposure conditions. In the present study, no information on these factors was obtained. Attempts were made to standardize these potential sources of variation by feeding all strains as nearly alike as possible and by choosing, for test purposes, adult snails of approximately the same size.

SUMMARY

Several laboratory strains of *Australorbis glabratus* were compared for their relative susceptibilities to the lethal action of sodium pentachlorophenate. A Venezuelan, a Puerto Rican, a Brazilian (Bwt), and a Brazilian albino mutant (Bm) strain derived from the latter, were used. Each strain had been maintained in the Laboratory for at least 3 years.

The estimated LD_{50}'s, at 24 hours and at a mean temperature of 80° F. (78°–84° F), for the Bm, Bwt, and Venezuelan strains were 0.68 ± .016, 0.80 ± .013, and 1.06 ± .020 p.p.m., respectively. Significantly different slopes were also obtained when probit lines were fitted to the dosage-mortality data for each strain. These findings indicated that these three strains of *A. glabratus* differed in average susceptibility and in homogeneity. On the basis of results with fewer tests, the dosage-response characteristics of the Puerto Rican strain appeared to be similar to those of the Venezuelan strain.

The relationship of these findings to the general problem of the control of *A. glabratus* with sodium pentachlorophenate is discussed.

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RESULTS OF LABORATORY SCREENING TESTS OF CHEMICAL COMPOUNDS FOR MOLLUSCICIDAL ACTIVITY

I. Phenols and Related Compounds

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INTRODUCTION

In June 1947, a screening program was initiated in these Institutes having as its goal the finding of chemical agents suitable for use in the destruction of freshwater snails that serve as intermediate hosts for the schistosomes and other trematode parasites of man. By late 1948, more than 80 compounds had been found which showed activity in varying degrees against *Australorbis glabratus*. It was noted that many of these were phenols or closely related compounds, including benzoquinones and naphthoquinones. In January 1949 and the spring of 1950 (Nolan *et al.*, 1949; Berry *et al.*, 1950), field trials were conducted in southern Texas and Puerto Rico with compounds selected as a result of the laboratory tests. These trials indicated that certain phenols showed considerable promise as molluscicides. The laboratory testing program has continued since that time and is still in progress.

One of the earliest reports to come to our attention on the successful use of a phenolic chemical as a molluscicide in the field was made by Humphreys (1932). He described methods and measures adopted to prevent the introduction and spread of schistosomiasis in a newly irrigated area in the Anglo-Egyptian Sudan. Humphreys stated that in the Gezira district, which lies in the triangle formed by the Blue and White Niles before they join at Khartoum, 487 miles of canals were treated with a commercial carbolic liquid "sizolin" used in the strength of 1 in 20,000. Within more recent years, other investigators have also indicated the high potentialities of certain phenolic compounds in the control of amphibious and aquatic vectors of the human schistosomes (3, 5, 6, 7, 8, 9, 10, 11). Subsequent to the completion of the major portion of our studies, Batte and Swanson (1952) reported on laboratory screening tests of a number of phenols against lymnaeid snails, freshwater hosts of the liver fluke of cattle in Florida. Their results were in substantial agreement with ours.

Of the approximately 1800 chemicals which we have tested to date, 701 are phenols or closely related compounds. This report deals with the results obtained with these 701 compounds. Subsequent articles will deal with the other compounds tested.

MATERIALS AND METHODS

Snails. In all of the screening tests, the freshwater pulmonate snail, *Australorbis glabratus*, vector of *Schistosoma mansoni* in endemic areas of the Western Hemi-

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sphere, was used as the test animal. It was easily raised and maintained in the laboratory once the difficulties attendant upon the initial buildup of a healthy laboratory-bred colony were overcome. On the basis of periodic tests with other species of snails, it proved to be one of the most resistant to toxic chemicals.

All test snails were the descendants of 10 "wild" *A. glabratus* kindly sent to us by Drs. J. Jove and A. Gabaldon of the Ministry of Health of Venezuela. They were constituted of numerous generations reared under conditions which were standardized before the actual testing of the chemicals got under way. These conditions were maintained with as little fluctuation as possible during the course of the screening program. This particular Venezuelan strain of *Australorbis* was the only one raised in this snailery; no new Venezuelan or any other strains of *Australorbis* were introduced into the colony. Promiscuous breeding in the group was allowed to occur, individual snails being transferred randomly from aquarium to aquarium. Young adult snails were used in the tests; they were removed from many tanks at the time that they were needed and the numbers required for the tests taken from the pooled lot.

The snails were reared in glass aquaria of 8 to 10 gallon capacity. The bottoms of the aquaria were covered with fine gravel to a depth of approximately one inch; the aquaria were filled with tap water[3] (pH 7.6 to 8.0) and mechanically aerated, air releasers being used to obtain a fine spray of bubbles. The plants in the aquaria were mainly *Cabomba*, *Elodea*, *Vallisneria*, and water sprite. Each aquarium was fitted with a glass cover which slowed the evaporation of the water and prevented the accidental dropping of foreign material into the tank. Fluorescent electric lamps, hung from the ceiling to a distance of two feet above the aquaria, provided the necessary light. Lighting was continuous except for three nights a week.

At least 50 aquaria were maintained in order that a constant supply of snails of the right age and size would be available for the tests. Optimum room temperature for snail growth and reproduction was found to be around 27° C. This temperature was maintained in the snailery during the winter by supplementing the heat derived from the central heating system of the building with a small portable heating unit having thermostat control, and during the summer with an air conditioner. Under these conditions, there were steady growth and reproduction with no apparent seasonal variation in the number of snails.

It has been our experience that 300 to 400 snails, ranging from newly-hatched juveniles to large-sized adults, can be maintained successfully in an 8-gallon aquarium, provided ample food is available and the mineral needs of the snails are fulfilled. The food of the snails consisted of fresh green lettuce leaves (romaine lettuce) and a finely-particled tropical fish food, in addition to the algae and other organisms that are normally available in a well-planted and seasoned aquarium. The dried food was made available to the snails for several hours daily by scattering it over the surface of the water after the air stream had been turned off. This latter precaution prevented the food from being churned about and dropping to the bottom where in excess amount it would quickly foul

[3] Tap water which was dechlorinated by standing in large glass cylinders for at least 24 hours, usually 48 hours or longer.

the tank. Fresh lettuce leaves were placed in each aquarium as a daily routine, and two or three times a week residual pieces were removed.

"Neutralizer bricks"[4] containing mineral salts and vitamins were kept in the aquaria. Aquarium water was siphoned off and replenished whenever needed, on the average once every two months. A complete aquarium overhaul was made about three times a year. Supplemental calcium in the form of calcium carbonate[5] was added to the aquaria at more or less regular intervals (twice a week proved optimal). The powdered calcium salt was mixed with an equal amount of water in a stoppered container, shaken thoroughly, and two to three tablespoons of the mixture poured into each aquarium. The salt was dispersed throughout the aquarium by the air stream and gradually settled on the greenery and the gravel at the bottom where it was consumed by the snails. Under the conditions existing in our setup, supplemental calcium appeared to be indispensable for the rearing of snails with strong, unimpaired shells, essential for any tests involving chemical compounds.

Experimental procedures. Since *Australorbis glabratus* is an aquatic snail and can not survive for long periods out of water, it was necessary to test all chemicals in aqueous solutions. Standing tap water, such as that used in the aquaria in which the snails were reared, was employed throughout the experiments.

Compounds were tested as received from the supplier without any attempts at purification or confirmation of structure. They were diluted in volumetric flasks with water to the desired parts per million (ppm) from freshly prepared 1, 0.5, or 0.25 per cent solutions (weight/volume) in acetone, ethyl alcohol or water, used singly or as co-solvents. Compounds that did not dissolve readily were placed on a shaker with glass beads for varying periods of time. Tween 80[6] was used to aid in the solution of difficultly soluble chemicals. In the case of practically insoluble chemicals, fine suspensions of necessity were used. Control tests showed that the solvents in the dilutions employed were completely nontoxic to snails.

Standards for the screening tests were as follows: concentration of chemical, 10 ppm; number of snails per test, 10 (5 snails being immersed in 250 ml. of the chemical solution in each of two beakers of 600 ml. capacity); exposure period, 24 hours. Criteria of effectiveness were based on determinations of death or survival of snails following their transfer from the chemically treated water into fresh water. Gentle aeration was provided at all times. Room temperatures were generally 26 to 27° C., with occasional temporary fluctuations ranging from 25 to 30° C.

At the end of each contact period, the snails were removed from the chemically treated water, washed thoroughly and transferred to aerated fresh water. Food (lettuce leaves) was made available to the snails. On the following day mortality rates were determined by microscopic examination. If snails were moribund or

[4] A commercial aquarium product stated to contain calcium, copper, iodine, iron, magnesium, manganese, phosphorus, zinc, and vitamins A, B_1, B_2, C, and D.

[5] Other calcium salts might have proved equally satisfactory but were not investigated.

[6] A polyoxyalkylene derivative of sorbitan monooleate, product of Atlas Powder Company.

had not fully recovered at the time of this examination they were held for further daily observations until death or revival occurred. Only in a minority of cases was it necessary to prolong the observation period beyond the one examination. Criteria for death were absence of heartbeat, if the heart could be detected, and lack of response to gentle prodding with a curved blunt needle that could be inserted into the curved shells of the snails, as far back as the inner whorls if the bodies of the snails were so deeply retracted. Determination of recovery was based on the ability of the snail to move about or cling to the bottom and sides of the glass container.

We would like to mention here that observations were also made on the condition of the snails and their reactions to the chemicals during and immediately following the contact periods. The reactions detected were limited and ran the gamut of little or deep retraction, slight or marked (rarely) extension of the body beyond the pallial edge of the shell, swollen tissues, destruction of the tentacles, hemorrhaging, excess mucus, simple inertia. Some of the compounds acted as irritants, causing the snails to attempt to crawl out of the chemically treated water. At the termination of the 24-hour exposure period, the number of snails found dead was also recorded. The data thus assembled may be of value in correlating field trials with laboratory tests of chemicals.

For every group of chemicals tested on any given day, 10 control snails were maintained unfed in two beakers in untreated water for the same periods of time as the test snails. In the hundreds of tests completed there were only 3 deaths among the control snails.

RESULTS

In the lists which follow the results obtained with each of the compounds are recorded. The symbol in column 2 refers to the supplier (see key below). In column 3 is given the empirical formula and in column 4 the name of the compound. In many cases, the names have been deliberately chosen to emphasize the fact that the compound is a phenol. In the last column are mortality rates (percentages) based on the number of snails determined as dead following their transfer to fresh water after the 24-hour exposure to 10 ppm of chemical.

In numerous instances, repeat tests of chemicals were made. For the most part the results were identical. A chemical received from different sources sometimes gave different results. In the case of extremely insoluble compounds erratic results were often obtained, obviously due to failure of the compounds to reach the test animals. Where there was variation in mortality rates the data of the various tests are all listed (column 5).

The division of the compounds is for convenience and is arbitrary. Many compounds could easily be placed in more than one grouping.

KEY TO SUPPLIERS OF CHEMICALS

SYMBOL	SUPPLIER
A	Allied Chemical and Dye Corporation
Ab	Abbott Laboratories
Ad	Adlershauf, Berlin
An	Antimalarial Drug Repository, NIH

B	Biosol Products Company
Ba	J. T. Baker Chemical Company
C	Chemical-Biological Coordination Center, National Research Council
Ca	Carbide and Carbon Chemicals Company
Co	Coleman and Bell Company
D	Dow Chemical Company
Du	E. I. du Pont de Nemours and Company
E	Eastman Kodak Company
Ed	Edwal Laboratories, Inc.
Ei	Eimer and Amend (Fisher Scientific Company)
El	Elko Company
F	Firestone Tire and Rubber Corporation
G	Gulf Research and Development Company
Ge	Genesee Research Corporation
H	Harvard University (Dr. L. F. Fieser); Western Reserve University (Dr. E. Bueding)
He	Heyden Chemical Corporation
Hl	Hartman-Leddon Company
Ho	Hoffman and Kropff Chemical Company
Hy	Hynson, Westcott and Dunning, Inc.
I	Irvington Varnish and Insulator Company
Il	Illinois State Geological Survey (Dr. G. C. Finger)
K	Koppers Company, Inc.
M	Monsanto Chemical Company
Ma	Mallinckrodt Chemical Works
Me	Merck and Company, Inc.
Mi	Michigan State College (Dr. R. C. Huston)
Mk	A. D. MacKay
N	Neville Company
Na	National Aniline and Chemical Company, Inc.
Ny	New York Quinine and Chemical Works
P	Parke, Davis and Company
Pa	Paragon Testing Laboratories
Pf	Pfanstiehl Chemical Company
R	Rohm and Haas Company
S	Sharples Chemical Company, Inc.
Si	Sindar Corporation
Sq	E. R. Squibb and Sons
Sy	Syn-Zyme Laboratories
T	W. A. Taylor and Company, Inc.
Tr	Tropical Diseases Laboratory, NIH
U	University of Maryland (Dr. W. R. Boehme)
W	Winthrop-Stearns, Inc.

LIST OF CHEMICALS TESTED

I. Phenol

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
1	—	C_6H_6O	Phenol	0
		II. Alkylated phenols and closely related compounds		
2	E	C_7H_8O	2-Methylphenol (*o*-Cresol)	0
3	E	C_7H_8O	3-Methylphenol (*m*-Cresol)	0
4	E	C_7H_8O	4-Methylphenol (*p*-Cresol)	0
5	F	$C_8H_{10}O$	2,4-Dimethylphenol (*unsym-m*-Xylenol)	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
6	E	$C_8H_{10}O$	2,5-Dimethylphenol (*p*-Xylenol)	0
7	F	$C_8H_{10}O$	2,6-Dimethylphenol (*vic-m*-Xylenol)	0
8	E	$C_8H_{10}O$	3,4-Dimethylphenol (*unsym-o*-Xylenol)	0
9	E	$C_8H_{10}O$	3-Ethylphenol	0
10	E	$C_8H_{10}O$	4-Ethylphenol	0
11	E	$C_9H_{12}O$	2,3,5-Trimethylphenol	0
12	F	$C_9H_{12}O$	2,3,6-Trimethylphenol	0
13	F	$C_9H_{12}O$	2,4,6-Trimethylphenol	0
14	F	$C_9H_{12}O$	3,4,5-Trimethylphenol	0
15	E	$C_9H_{12}O$	3-Ethyl-5-methylphenol	0
16	D	$C_9H_{12}O$	2-Isopropylphenol	0
17	D	$C_9H_{12}O$	4-Isopropylphenol	0
18	F	$C_{10}H_{14}O$	2,3,5,6-Tetramethylphenol	0
19	E	$C_{10}H_{14}O$	5-Isopropyl-2-methylphenol	0
20	Sq	$C_{10}H_{14}O$	2-Isopropyl-5-methylphenol	0
21	D	$C_{10}H_{14}O$	4-*tert*-Butylphenol	0
22	F, K	$C_{11}H_{16}O$	2-*tert*-Butyl-4-methylphenol	20, 70
23	K	$C_{11}H_{16}O$	2-*tert*-Butyl-5-methylphenol	20
24	K	$C_{11}H_{16}O$	2-*tert*-Butyl-6-methylphenol	0
25	S	$C_{11}H_{16}O$	2-Amylphenol	10
26	E	$C_{11}H_{16}O$	4-*tert*-Amylphenol	0
27	F	$C_{12}H_{18}O$	6-*tert*-Butyl-2,4-dimethylphenol	0
28	K	$C_{12}H_{18}O$	2-Amyl-5-methylphenol	0, 40
29	K	$C_{13}H_{20}O$	6-*tert*-Butyl-2-isopropylphenol	0
30	D	$C_{14}H_{22}O$	2,4-Di-*sec*-butylphenol	80
31	R	$C_{14}H_{22}O$	4-*tert*-Octylphenol	100
32	K	$C_{15}H_{24}O$	2,4-Di-*tert*-butyl-5-methylphenol	90
33	F, K	$C_{15}H_{24}O$	2,4-Di-*tert*-butyl-6-methylphenol	0
34	E, K	$C_{15}H_{24}O$	2,6-Di-*tert*-butyl-4-methylphenol	0
35	K	$C_{15}H_{24}O$	4-Nonylphenol	40*, 70†
36	F	$C_{16}H_{26}O$	2,4-Dimethyl-6-octylphenol	0
37	E	$C_{16}H_{26}O$	2,4-Di-*sec*-amylphenol	80
38	E	$C_{16}H_{26}O$	2,4-Di-*tert*-amylphenol	10
39	K	$C_{17}H_{28}O$	2,4-Di-*tert*-butyl-6-isopropylphenol	0
40	K	$C_{17}H_{28}O$	2,4-Diamyl-5-methylphenol	50
41	F	$C_{18}H_{30}O$	2,6-Di-*tert*-butyl-4-*sec*-butylphenol	0
42	F	$C_{18}H_{30}O$	2,4,6-Tri-*tert*-butylphenol	0
43	S	$C_{18}H_{30}O$	*x*-Dodecylphenol	20
44	I	$C_{21}H_{36}O$	3-Pentadecylphenol	0
45	F	$C_{22}H_{40}O$	2,6-Dioctyl-4-methylphenol	0
46	C	$C_9H_{10}O$	2-Allylphenol	0
47	C	$C_{10}H_{12}O$	2-Allyl-6-methylphenol	0
48	I	$C_{21}H_{32}O$	3-Pentadecadienylphenol	0
49	E	$C_{12}H_{16}O$	2-Cyclohexylphenol	80
50	D	$C_{12}H_{16}O$	4-Cyclohexylphenol	70
51	F	$C_{16}H_{22}O$	2-Isobornylphenol	100

* Nonylphenol "B"

† Nonylphenol "A"

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
52	F	$C_{17}H_{26}O$	2-Isobornyl-4-methylphenol	100
53	F	$C_{18}H_{28}O$	2-Isobornyl-4,6-dimethylphenol	0
54	F	$C_{27}H_{40}O$	2,6-Diisobornyl-4-methylphenol	0
55	C	$C_7H_8O_2$	2-Hydroxymethylphenol (2-Hydroxybenzyl alcohol; saligenin)	0
56	C	$C_8H_8O_3$	4-Carboxymethylphenol (4-Hydroxyphenylacetic acid)	0
57	E	$C_9H_{11}NO_3$	L-Tyrosine	0
58	E	$C_{11}H_{15}NO_3$	L-Tyrosine, ethyl ester	0
59	E	$C_{10}H_{10}O_2$	Salicylideneacetone	0
60	E	$C_{10}H_8N_2O_2S$	5-(2′-Hydroxybenzal)-2-thiohydantoin	0
61	C	$C_{12}H_{11}NO$	2-Hydroxybenzalaniline (α-Phenylimino-*o*-cresol)	0
62	C	$C_{16}H_{17}NO_2$	2-Hydroxybenzal-4-phenetidine (α-(*p*-Phenethylimino)-*o*-cresol)	0
63	R	$C_{18}H_{27}N_3O$	2,4,6-Tris(dimethylaminomethyl) phenol	0
64	R	$C_{17}H_{29}NO$	2-Dimethylaminomethyl-4-octylphenol	0
65	P	$C_{21}H_{33}N_3O_4$	2,4,6-Tris(4′-morpholinylmethyl) phenol	0
		III. Halogenated phenols and closely related compounds		
66	E	C_6H_5BrO	2-Bromophenol	0
67	—	C_6H_5BrO	3-Bromophenol	0
68	Ho	C_6H_5BrO	4-Bromophenol	0
69	E	C_6H_5ClO	2-Chlorophenol	0
70	E	C_6H_5ClO	3-Chlorophenol	0
71	E, M	C_6H_5ClO	4-Chlorophenol	0
72	Il	C_6H_5FO	2-Fluorophenol	0
73	Il	C_6H_5FO	3-Fluorophenol	0
74	Il	C_6H_5FO	4-Fluorophenol	0
75	E	C_6H_5IO	2-Iodophenol	0
76	K	C_7H_5NOS	4-Thiocyanophenol	40
77	E	$C_6H_4Br_2O$	2,4-Dibromophenol	60
78	—	$C_6H_4Br_2O$	2,5-Dibromophenol	100
79	E	$C_6H_4Br_2O$	2,6-Dibromophenol	100
80	E, M	$C_6H_4Cl_2O$	2,4-Dichlorophenol	0
81	E	$C_6H_4Cl_2O$	2,5-Dichlorophenol	0
82	El	$C_6H_4Cl_2O$	2,6-Dichlorophenol	0
83	E	$C_6H_3Br_3O$	2,4,6-Tribromophenol	100
84	D	$C_6H_3Cl_3O$	2,3,6-Trichlorophenol	50
85	M	$C_6H_3Cl_3O$	2,4,5-Trichlorophenol	100
86	E	$C_6H_3Cl_3O$	2,4,6-Trichlorophenol	100
87	E, Ei	$C_6H_3I_3O$	2,4,6-Triiodophenol	100
88	D	$C_6H_2Cl_4O$	2,3,4,6-Tetrachlorophenol	100
89	D, E	C_6HBr_5O	Pentabromophenol	100
90	D, E, M	C_6HCl_5O	Pentachlorophenol	100
91	C	$C_6H_3BrCl_2O$	4-Bromo-2,6-dichlorophenol	90
92	C	$C_6H_3Br_2ClO$	2,6-Dibromo-4-chlorophenol	100

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
93	C	$C_6H_3ClI_2O$	4-Chloro-2,6-diiodophenol	100
94	D	C_6HBrCl_4O	3-Bromo-2,4,5,6-tetrachlorophenol	100
95	D	$C_6HBr_2Cl_3O$	3,5-Dibromo-2,4,6-trichlorophenol	100
		IV. Nitro-, amino- and nitrosophenols		
96	C	$C_6H_5NO_3$	2-Nitrophenol	0
97	Ad	$C_6H_5NO_3$	3-Nitrophenol	0
98	E	$C_6H_5NO_3$	4-Nitrophenol	0
99	C	$C_6H_4N_2O_5$	2,4-Dinitrophenol	0
100	E	$C_6H_4N_2O_5$	2,6-Dinitrophenol	0
101	—	$C_6H_3N_3O_7$	2,4,6-Trinitrophenol (Picric acid)	0
102	E	$C_6H_5NO_2$	4-Nitrosophenol	0
103	E	C_6H_7NO	2-Aminophenol	0
104	E	C_6H_7NO	3-Aminophenol	0
105	E	C_6H_7NO	4-Aminophenol	70, 100
106	E	$C_6H_7NO \cdot HCl$	4-Aminophenol hydrochloride	30
107	E	$C_6H_8N_2O \cdot 2HCl$	2,4-Diaminophenol dihydrochloride	0
108	C	$C_6H_9N_3O \cdot 3HCl$	2,4,6 Triaminophenol trihydrochloride	0
109	E	$C_6H_6N_2O_3$	2-Amino-4-nitrophenol	0
110	E	$C_6H_5N_3O_5$	2-Amino 4,6-dinitrophenol (Picramic acid)	0
111	E	$C_8H_9NO_2$	2-Acetylaminophenol	0
112	E	$C_8H_9NO_2$	3-Acetylaminophenol	0
113	E	$C_8H_9NO_2$	4-Acetylaminophenol	0
114	C	$C_{10}H_{12}NO_3$	4-Hydroxycarbanilic acid, isopropyl ester	0
115	Ny	$C_7H_9NO \cdot \frac{1}{2}H_2SO_4$	4-Methylaminophenol sulfate	100
116	E	$C_9H_{11}NO_2$	*N*-Acetyl-2-methylaminophenol	0
117	E	$C_9H_{11}NO_2$	*N*-Acetyl-4-methylaminophenol	0
118	E	$C_8H_{11}NO \cdot \frac{1}{2}H_2SO_4$	4-Dimethylaminophenol sulfate	90
119	C	$C_8H_{11}NO \cdot \frac{1}{2}(COOH)_2$	4-Dimethylaminophenol oxalate	60
120	E	$C_{10}H_{15}NO$	3-Diethylaminophenol	0
121	E	$C_8H_9NO_3$	4-Hydroxyphenylglycine	70
122	E	$C_{10}H_{15}NO_2$	4-Di-(β-hydroxyethyl)aminophenol	60
123	C	$C_{12}H_{11}NO$	4-Phenylaminophenol (*p*-Anilinophenol)	30
124	E	$C_{13}H_{12}NO$	4-Benzylaminophenol	70
125	C	$C_{16}H_{13}NO$	4-β-Naphthylaminophenol	90*
126	C, E	$C_{13}H_{11}NO$	4-Benzalaminophenol	20
		V. Alkylated-halogenated phenols		
127	K	C_7H_7ClO	2-Chloro-4-methylphenol	0
128	C	C_7H_7ClO	2-Chloro-6-methylphenol	0
129	C	C_7H_7ClO	4-Chloro-2-methylphenol	0

*48-hour exposure period.

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
130	E, M	C_7H_7ClO	4-Chloro-3-methylphenol	0
131	K	C_8H_9ClO	2-Chloro-3,5-dimethylphenol	0
132	K	C_8H_9ClO	4-Chloro-2,6-dimethylphenol	0
133	M	C_8H_9ClO	4-Chloro-3,5-dimethylphenol	0
134	K	C_8H_9ClO	2- and 6-Chloro-3,4-dimethylphenol	0
135	K	C_8H_9ClO	2-Chloro-4-ethylphenol	10
136	K	C_8H_9ClO	4-Chloro-3-ethylphenol	0
137	K	C_8H_9ClO	2- and 6-Chloro-3-ethylphenol	0
138	K	$C_9H_{11}ClO$	2-Chloro-4-isopropylphenol	0
139	K	$C_9H_{11}ClO$	2-Chloro-6-isopropylphenol	0
140	K	$C_9H_{11}ClO$	3-Chloro-4-isopropylphenol	0
141	K	$C_9H_{11}ClO$	4-Chloro-2-isopropylphenol	80
142	K	$C_9H_{11}ClO$	4-Chloro-3-isopropylphenol	0
143	E	$C_{10}H_{13}ClO$	2-Chloro-4-*tert*-butylphenol	10
144	K	$C_{10}H_{13}ClO$	2-Chloro-3-isopropyl-6-methylphenol	0
145	K	$C_{10}H_{13}ClO$	4-Chloro-2-isopropyl-5-methylphenol	60
146	K	$C_{10}H_{13}ClO$	4-Chloro-3-isopropyl-5-methylphenol	60
147	K	$C_{10}H_{13}ClO$	4-Chloro-5-isopropyl-2-methylphenol	50
148	S	$C_{11}H_{15}ClO$	2-Chloro-4-*tert*-amylphenol	30
149	K	$C_{11}H_{15}ClO$	2-Chloro-6-amylphenol	0
150	K	$C_{11}H_{15}ClO$	2-Chloro-4-*tert*-butyl-6-methylphenol	0
151	K	$C_{11}H_{15}ClO$	2-Chloro-6-*tert*-butyl-4-methylphenol	0
152	K	$C_{11}H_{15}ClO$	4-Chloro-2-amylphenol	60
153	K	$C_{11}H_{15}ClO$	4-Chloro-2-*tert*-butyl-5-methylphenol	50
154	K	$C_{11}H_{15}ClO$	4-Chloro-2-*tert*-butyl-6-methylphenol	0
155	K	$C_{11}H_{15}ClO$	2- and 6-Chloro-4-*tert*-butyl-3-methylphenol	0
156	K	$C_{12}H_{17}ClO$	2-Chloro-4,6-diisopropylphenol	0
157	K	$C_{14}H_{21}ClO$	2-Chloro-4-octylphenol	100
158	F	$C_{14}H_{21}ClO$	4-Chloro-2,6-di-*tert*-butylphenol	0
159	K	$C_{15}H_{23}ClO$	2-Chloro-4-nonylphenol	10
160	K	$C_{15}H_{23}ClO$	2-Chloro-4,6-di-*tert*-butyl-3-methylphenol	0
161	K	$C_{16}H_{25}ClO$	2-Chloro-4,6-diamylphenol	0
162	S	$C_{16}H_{25}ClO$	2-Chloro-4,6-di-*tert*-amylphenol	0
163	K	$C_{21}H_{35}ClO$	4-Chloro-3-pentadecylphenol	0
164	K	$C_{12}H_{15}ClO$	2-Chloro-4-cyclohexylphenol	90
165	K	$C_{12}H_{15}ClO$	2-Chloro-6-cyclohexylphenol	0
166	K	$C_{12}H_{15}ClO$	4-Chloro-2-cyclohexylphenol	100
167	P	$C_{13}H_{18}ClNO_2$	4-Chloro-3,5-dimethyl-2-(4′-morpholinylmethyl)phenol	0
168	P	$C_{14}H_{20}ClNO$	4-Chloro-3,5-dimethyl-2-(1′-piperidinylmethyl)phenol	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
169	C	C_8H_9BrO	4-Bromo-3,5-dimethylphenol	0
170	E	$C_{10}H_{13}BrO$	2-Bromo-4-*tert*-butylphenol	90
171	C	$C_7H_7BrO_2$	5-Bromo-2-hydroxybenzylalcohol	0
172	Ma	$C_{10}H_{13}IO$	4-Iodo-2-isopropyl-5-methylphenol	0
173	K	C_8H_7NOS	4-Thiocyano-2-methylphenol	0, 30
174	K	C_8H_7NOS	4-Thiocyano-3-methylphenol	20
175	K	C_9H_9NOS	4-Thiocyano-2,6-dimethylphenol	20
176	K	C_9H_9NOS	4-Thiocyano-3,5-dimethylphenol	80
177	K	C_9H_9NOS	4-Thiocyano-2-ethylphenol	70, 80
178	K	C_9H_9NOS	4-Thiocyano-3-ethylphenol	60
179	K	$C_{10}H_{11}NOS$	4-Thiocyano-2-isopropylphenol	80
180	K	$C_{11}H_{13}NOS$	4-Thiocyano-2-isopropyl-5-methylphenol	70
181	K	$C_{12}H_{15}NOS$	4-Thiocyano-2-amylphenol	90, 100
182	K	$C_{12}H_{15}NOS$	4-Thiocyano-2-*tert*-butyl-5-methylphenol	100
183	K	$C_{12}H_{15}NOS$	4-Thiocyano-2-*tert*-butyl-6-methylphenol	20
184	E	$C_7H_6Br_2O$	2,4-Dibromo-6-methylphenol	10
185	C	$C_7H_6Br_2O$	2,6-Dibromo-4-methylphenol	0
186	D	$C_{10}H_{12}Br_2O$	2,6-Dibromo-4-*tert*-butylphenol	70
187	M	$C_7H_6Cl_2O$	2,4-Dichloro-5-methylphenol	50
188	K	$C_7H_6Cl_2O$	2,4-Dichloro-6-methylphenol	0
189	K	$C_7H_6Cl_2O$	2,6-Dichloro-4-methylphenol	0
190	K	$C_7H_6Cl_2O$	2,4-, 2,6-, and 4,6-Dichloro-3-methylphenol	0
191	K	$C_8H_8Cl_2O$	2,4-Dichloro-3,5-dimethylphenol	20
192	K	$C_8H_8Cl_2O$	2,5-, 2,6-, and 5,6-Dichloro-3,4-dimethylphenol	0
193	K	$C_8H_8Cl_2O$	2,4-Dichloro-3-ethylphenol	80
194	K	$C_8H_8Cl_2O$	2,6-Dichloro-4-ethylphenol	20
195	K	$C_9H_{10}Cl_2O$	2,4-Dichloro-6-isopropylphenol	10
196	K	$C_9H_{10}Cl_2O$	2,6-Dichloro-4-isopropylphenol	60
197	K	$C_{10}H_{12}Cl_2O$	2,4-Dichloro-6-isopropyl-3-methylphenol	0
198	K	$C_{10}H_{12}Cl_2O$	2,6-Dichloro-4-*tert*-butylphenol	80
199	K	$C_{10}H_{12}Cl_2O$	2,3- and 2,5-Dichloro-4,6-diethylphenol	0
200	K	$C_{10}H_{12}Cl_2O$	2,4-, 2,6-, and 4,6-Dichloro-3-isopropyl-5-methylphenol	80
201	K	$C_{11}H_{14}Cl_2O$	2,4-Dichloro-6-*tert*-butyl-3-methylphenol	0
202	K	$C_{11}H_{14}Cl_2O$	2,4-Dichloro-6-amylphenol	0
203	K	$C_{11}H_{14}Cl_2O$	2,3- and 2,5-Dichloro-6-*tert*-butyl-4-methylphenol	0
204	K	$C_{11}H_{14}Cl_2O$	2,6-Dichloro-4-*tert*-amylphenol	100
205	K	$C_{12}H_{16}Cl_2O$	2,3- and 2,5-Dichloro-4,6-diisopropylphenol	0
206	K	$C_{12}H_{18}Cl_2O$	2,4-Dichloro-3-isopropyl-6-*tert*-butylphenol	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
207	K	$C_{14}H_{20}Cl_2O$	2,6-Dichloro-4-octylphenol	100
208	K	$C_{15}H_{22}Cl_2O$	2,6-Dichloro-4-nonylphenol	40
209	K	$C_{21}H_{34}Cl_2O$	2,4- and 2,6-Dichloro-3-pentadecylphenol	0
210	K	$C_{12}H_{14}Cl_2O$	2,4-Dichloro-6-cyclohexylphenol	80
211	K	$C_{12}H_{14}Cl_2O$	2,6-Dichloro-4-cyclohexylphenol	70
212	C	$C_7H_6I_2O$	2,4-Diiodo-6-methylphenol	50
213	E	$C_9H_9I_2NO_3$	3,5-Diiodo-L-tyrosine	0
214	M	$C_8H_7Cl_3O$	2,4,6-Trichloro-3,5-dimethylphenol	90
215	E	$C_7H_4Br_4O$	2,3,4,5-Tetrabromo-6-methylphenol	100
216	M	$C_7H_4Cl_4O$	2,3,4,6-Tetrachloro-5-methylphenol	100
			VI. Alkyl-nitro- and alkyl-aminophenols	
217	K	$C_7H_7NO_3$	2-Nitro-5-methylphenol	0
218	K	$C_7H_7NO_3$	2-Nitro-6-methylphenol	0
219	K	$C_7H_7NO_3$	4-Nitro-2-methylphenol	0
220	K	$C_7H_7NO_3$	4-Nitro-3-methylphenol	0
221	K	$C_8H_9NO_3$	2-Nitro-3,5-dimethylphenol	0
222	K	$C_8H_9NO_3$	2-Nitro-4,5-dimethylphenol	0
223	K	$C_8H_9NO_3$	2-Nitro-4,6-dimethylphenol	0
224	K	$C_8H_9NO_3$	4-Nitro-2,6-dimethylphenol	40
225	K	$C_8H_9NO_3$	2-Nitro-5-ethylphenol	0
226	K	$C_8H_9NO_3$	4-Nitro-3-ethylphenol	0
227	K	$C_9H_{11}NO_3$	2-Nitro-5-isopropylphenol	20
228	I	$C_{21}H_{35}NO_3$	2-Nitro-5-pentadecylphenol	0
229	I	$C_{21}H_{35}NO_3$	4-Nitro-3-pentadecylphenol	0
230	K	$C_7H_6N_2O_5$	2,4-Dinitro-5-methylphenol	0
231	E	$C_7H_6N_2O_5$	2,4-Dinitro-6-methylphenol	50
232	K	$C_8H_8N_2O_5$	2,4-Dinitro-3,5-dimethylphenol	30
233	K	$C_8H_8N_2O_5$	2,4-Dinitro-3,6-dimethylphenol	60
234	K	$C_8H_8N_2O_5$	2,4-Dinitro-5-ethylphenol	0
235	K	$C_9H_{10}N_2O_5$	2,4-Dinitro-3-methyl-5-ethylphenol	100
236	D	$C_9H_{10}N_2O_5$	2,4-Dinitro-6-isopropylphenol	100
237	E, K	$C_{10}H_{12}N_2O_5$	2,4-Dinitro-3-methyl-6-isopropylphenol	90, 100
238	D	$C_{10}H_{12}N_2O_5$	2,4-Dinitro-6-*sec*-butylphenol (Dinitro-*o*-*sec*-butylphenol)	100
239	K	$C_{11}H_{14}N_2O_5$	2,4-Dinitro-3-methyl-6-*tert*-butylphenol	100
240	K	$C_{11}H_{14}N_2O_5$	2,4-Dinitro-6-*sec*-amylphenol	100
241	I	$C_{21}H_{34}N_2O_5$	2,4-Dinitro-3 pentadecylphenol	0
242	I	$C_{21}H_{34}N_2O_5$	2,4 Dinitro-5-pentadecylphenol	0
243	D	$C_{12}H_{14}N_2O_5$	2,4-Dinitro-6-cyclohexylphenol (Dinitro-*o*-cyclohexylphenol)	100
244	D	$C_{12}H_{14}N_2O_5 \cdot (C_6H_{11})_2NH$	2,4-Dinitro-6-cyclohexylphenol, dicyclohexylamine salt	100
245	K	$C_7H_6N_2O_5$	2,6 Dinitro-4-methylphenol	30

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
246	K	$C_8H_8N_2O_5$	2,6-Dinitro-3,4-dimethylphenol	10
247	K	$C_8H_8N_2O_5$	2,6-Dinitro-4-ethylphenol	0
248	K	$C_9H_{10}N_2O_5$	2,6-Dinitro-4-isopropylphenol	20
249	K	$C_{10}H_{12}N_2O_5$	2,6-Dinitro-4-*tert*-butylphenol	40
250	K	$C_{11}H_{14}N_2O_5$	2,6-Dinitro-4-*sec*-amylphenol	100
251	K	$C_{11}H_{14}N_2O_5$	2,6-Dinitro-4-*tert*-amylphenol	50
252	K	$C_{12}H_{14}N_2O_5$	2,6-Dinitro-4-cyclohexylphenol	90
253	K	$C_7H_5N_3O_7$	2,4,6-Trinitro-3-methylphenol	0
254	K	$C_8H_7N_3O_7$	2,4,6-Trinitro-3-ethylphenol	0
255	I	$C_{21}H_{33}N_3O_7$	2,4,6-Trinitro-3-pentadecylphenol	0
256	K	$C_7H_7NO_2$	4-Nitroso-2-methylphenol	60
257	K	$C_7H_7NO_2$	4-Nitroso-3-methylphenol	0
258	K	$C_8H_9NO_2$	4-Nitroso-3,5-dimethylphenol	0
259	K	$C_8H_9NO_2$	4-Nitroso-3-ethylphenol	0
260	E, K	$C_{10}H_{13}NO_2$	4-Nitroso-2 isopropyl-5-methylphenol	30, 50
261	K	$C_{10}H_{13}NO_2$	4-Nitroso-5-isopropyl-2-methylphenol	0
262	K	$C_{11}H_{15}NO_2$	4-Nitroso 2 *tert*-butyl-5-methylphenol	50
263	K	$C_{11}H_{15}NO_2$	4-Nitroso-2-*tert*-butyl-6-methylphenol	0
264	K	$C_{13}H_{19}NO_2$	4-Nitroso-2-*tert*-butyl-6-isopropylphenol	10
265	I	$C_{21}H_{35}NO_2$	4-Nitroso-3-pentadecylphenol	0
266	E	$C_{21}H_{37}NO$	4-Amino-3-pentadecylphenol	0
			VII. Halogenated-nitrophenols	
267	E	$C_6H_4ClNO_3$	2-Chloro-4-nitrophenol	70
268	D	$C_6H_3ClN_2O_5$	2-Chloro-4,6-dinitrophenol	0
269	E	$C_6H_3ClN_2O_5$	4-Chloro-2,6-dinitrophenol	0
270	E	$C_6H_3Br_2NO_3$	2,6-Dibromo-4-nitrophenol	100
271	E	$C_6H_3Cl_2NO_3$	2,6-Dichloro-4-nitrophenol	100
272	E	$C_6H_5Br_2NO$	2,6-Dibromo-4-aminophenol	10
273	D	$C_6H_2Cl_3NO_3$	3,4,6-Trichloro-2-nitrophenol	80
274	E	$C_7H_6ClNO_3$	4-Chloro-2-nitro-3-methylphenol	0
			VIII. Phenolic aldehydes, ketones and their derivatives	
275	D	$C_7H_6O_2$	2-Hydroxybenzaldehyde (Salicylaldehyde)	0
276	E	$C_7H_6O_2$	3-Hydroxybenzaldehyde	0
277	E	$C_7H_6O_2$	4-Hydroxybenzaldehyde	0
278	E	$C_7H_5BrO_2$	2-Hydroxy-5-bromobenzaldehyde (5-Bromosalicylaldehyde)	60
279	E	$C_7H_5NO_4$	2-Hydroxy-5-nitrobenzaldehyde (5-Nitrosalicylaldehyde)	20
280	E	$C_8H_8O_2$	2-Hydroxy-5-methylbenzaldehyde (*p-homo*-Salicylaldehyde)	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
281	E	$C_7H_7NO_2$	2-Hydroxybenzaldehyde oxime (Salicylaldoxime)	0
282	C	$C_7H_7NO_2$	4-Hydroxybenzaldehyde oxime	0
283	E	$C_8H_8O_2$	2-Hydroxyacetophenone	0
284	C	$C_8H_8O_2$	3-Hydroxyacetophenone	0
285	E	$C_9H_{10}O_2$	2-Hydroxypropiophenone	0
286	E	$C_9H_{10}O_2$	4-Hydroxypropiophenone	0
287	D	$C_{13}H_{10}O_2$	4-Hydroxybenzophenone	0
			IX. Hydroxyaromatic acids, and their esters and amides	
			A. Carboxylic acids	
288	Ma	$C_7H_6O_3$	2-Hydroxybenzoic acid (Salicylic acid)	0
289	E	$C_7H_6O_3$	3-Hydroxybenzoic acid	0
290	—	$C_7H_6O_3$	4-Hydroxybenzoic acid	0
291	E	$C_8H_8O_3$	Methyl 2-hydroxybenzoate (Methyl salicylate)	0
292	E	$C_{10}H_{12}O_3$	Isopropyl 2-hydroxybenzoate (Isopropyl salicylate)	0
293	E	$C_{11}H_{14}O_3$	*n*-Butyl 2-hydroxybenzoate (*n*-Butyl salicylate)	0
294	E	$C_{12}H_{16}O_3$	Isoamyl 2-hydroxybenzoate (Isoamyl salicylate)	0
295	E	$C_{13}H_{10}O_3$	Phenyl 2-hydroxybenzoate (Phenyl salicylate; salol)	0
296	E	$C_{14}H_{12}O_3$	Benzyl 2-hydroxybenzoate (Benzyl salicylate)	0
297	P	$C_7H_7NO_2$	2-Hydroxybenzamide (Salicylamide)	0
298	C	$C_{13}H_{11}NO_2$	*N*-Phenyl-2-hydroxybenzamide (*N*-Phenyl salicylamide; salicylanilide)	10
299	C	$C_{14}H_{13}NO_2$	*N*-2-Tolyl-2′-hydroxybenzamide (*N*-2-Tolyl salicylamide)	60
300	C	$C_{21}H_{15}NO_2$	3-Hydroxy-2-anthranilide	0
301	D	$C_7H_5BrO_3$	2-Hydroxy-5-bromobenzoic acid (5-Bromosalicylic acid)	0
302	P	$C_7H_4Cl_2O_3$	2-Hydroxy-3,5-dichlorobenzoic acid (3,5-Dichlorosalicylic acid)	0
303	E	$C_7H_4I_2O_3$	2-Hydroxy-3,5-diiodobenzoic acid (3,5-Diiodosalicylic acid)	10
304	E	$C_7H_5NO_5$	2-Hydroxy-3-nitrobenzoic acid (3-Nitrosalicylic acid)	0
305	E	$C_7H_5NO_5$	2-Hydroxy-5-nitrobenzoic acid (5-Nitrosalicylic acid)	0
306	E	$C_7H_4N_2O_7$	2-Hydroxy-3,5-dinitrobenzoic acid (3,5-Dinitrosalicylic acid)	0
307	D	$C_8H_8O_3$	2-Hydroxy-3-methylbenzoic acid (3-Methylsalicylic acid; *o*-Cresotinic acid)	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
308	I	$C_{22}H_{32}O_2$	2-Hydroxy-3-pentadecadienylbenzoic acid (3-Pentadecadienylsalicylic acid; Anacardic acid)	0
309	I	$C_{22}H_{36}O_3$	2-Hydroxy-6-pentadecylbenzoic acid (6-Pentadecylsalicylic acid)	0
310	He	$C_8H_8O_3$	Methyl 4-hydroxybenzoate	0
311	He	$C_9H_{10}O_3$	Ethyl 4-hydroxybenzoate	0
312	He	$C_{10}H_{12}O_3$	*n*-Propyl 4-hydroxybenzoate	0
313	E	$C_7H_4I_2O_3$	3,5-Diiodo-4-hydroxybenzoic acid	0
314	C	$C_8H_6O_5$	3-Hydroxyphthalic acid	0
			B. *Sulfonic acids*	
315	D	$C_6H_6O_4S$	Phenol 4-sulfonic acid	0
316	C	$C_6H_7NO_3S$	Phenol 4-sulfonamide	0
317	E	$C_6H_4Cl_2O_4S$	4,6-Dichlorophenol 2-sulfonic acid	0
318	E	$C_6H_7NO_4S$	2-Aminophenol 4-sulfonic acid	0
319	K	$C_8H_{10}O_4S$	4,5-Dimethylphenol 2-sulfonic acid (3,4-Xylenol 6-sulfonic acid)	0
320	K	$C_8H_{10}O_4S$	3,5-Dimethylphenol sulfonic acid	0
321	K	$C_8H_{10}O_4S$	2-Ethylphenol 4-sulfonic acid	10
322	K	$C_8H_{10}O_4S$	3-Ethylphenol *x*-sulfonic acid	0
323	K	$C_8H_{10}O_4S$	4-Ethylphenol 2-sulfonic acid	0
324	K	$C_{10}H_{14}O_4S$	4-*tert*-Butylphenol 2-sulfonic acid	0
325	K	$C_{10}H_{14}O_4S$	Diethylphenol sulfonic acid	0
326	K	$C_{11}H_{16}O_4S$	6-*tert*-Butyl-2-methylphenol 4-sulfonic acid	0
327	E	$C_{10}H_{14}O_4S$	2-Isopropyl-5-methylphenol 4-sulfonic acid (Thymolsulfonic acid)	0
328	K	$C_{11}H_{16}O_4S$	2-Amylphenol 4-sulfonic acid	0
329	K	$C_{11}H_{16}O_4S$	4-*tert*-Amylphenol 2-sulfonic acid	0
330	K	$C_{14}H_{22}O_4S$	4-Octylphenol 2-sulfonic acid	0
331	I	$C_{21}H_{36}O_4S$	3-Pentadecylphenol 4-sulfonic acid	0
332	K	$C_{12}H_{16}O_4S$	2-Cyclohexylphenol 4-sulfonic acid	0
333	K	$C_{12}H_{16}O_4S$	4-Cyclohexylphenol 2-sulfonic acid	10
			C. *Carboxylic-sulfonic acid*	
334	Ba	$C_7H_6O_6S$	2-Hydroxy-5-sulfonic benzoic acid (Sulfosalicylic acid)	0
			D. *Arsonic acid*	
335	E	$C_6H_7AsO_4$	4-Hydroxyphenyl arsonic acid	0

LIST OF CHEMICALS TESTED—(*Cont.*)

X. Alkoxyphenols

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
336	E	$C_7H_8O_2$	2-Methoxyphenol (Guaiacol)	0
337	E	$C_7H_8O_2$	3-Methoxyphenol	0
338	E	$C_7H_8O_2$	4-Methoxyphenol	0
339	Ge	$C_8H_{10}O_2$	2-Ethoxyphenol	0
340	E	$C_{13}H_{12}O_2$	4-Benzyloxyphenol	0
341	E	$C_8H_8O_4$	4-Hydroxyphenoxyacetic acid	0
342	M	$C_{10}H_7ClO_5$	β-Chloro-β-formyl-α-(3-hydroxyphenoxy)acrylic acid	0
343	E	$C_8H_{10}O_2$	2-Methoxy-4-methylphenol (Creosol)	0
344	E	$C_{10}H_{12}O_2$	2-Methoxy-4-allylphenol (Eugenol)	0
345	E	$C_{10}H_{12}O_2$	2-Methoxy-4-propenylphenol (Isoeugenol)	0
346	C	$C_{10}H_{14}O_2$	2-Methoxy-4-*n*-propylphenol	0
347	E	$C_8H_{10}O_2$	2-Methoxy-4-hydroxymethylphenol (Vanillyl alcohol)	0
348	E	$C_9H_{10}O_3$	2-Methoxy-4-acetylphenol	0
349	C	$C_9H_{10}O_4$	4-Hydroxy-3-methoxyphenylacetic acid (Homovanillic acid)	0
350	E	$C_9H_{10}O_5$	Syringic acid (Gallic acid dimethyl ether)	0
351	C	$C_{10}H_{10}O_4$	4-Hydroxy-3-methoxycinnamic acid (Ferulic acid)	0
352	E	$C_{11}H_{12}O_3$	4-Hydroxy-3-methoxybenzalacetone (Vanillalacetone)	0
353	M	$C_8H_8O_3$	2-Hydroxy-3-methoxybenzaldehyde (Orthovanillin)	0
354	Me	$C_8H_8O_3$	4-Hydroxy-3-methoxybenzaldehyde (Vanillin)	0
355	Me	$C_8H_8O_4$	4-Hydroxy-3-methoxybenzoic acid (Vanillic acid)	0
356	C	$C_7H_7BrO_2$	3-Methoxy-4-bromophenol	0
357	C	$C_8H_7Br_3O_3$	2,6-Dimethoxy-3,4,5-tribromophenol	20
358	C	$C_{10}H_{12}O_2$	4-Methoxy-2-allylphenol	0
359	C	$C_{13}H_{16}O_2$	4-Methoxy-2,6-diallylphenol	0
360	F	$C_{15}H_{24}O_2$	4-Methoxy-2,6-di-*tert*-butylphenol	0
361	M	$C_{11}H_{14}O_2$	2-Ethoxy-6-allylphenol	0
362	M	$C_{11}H_{14}O_2$	2-Ethoxy-6-propenylphenol	0
363	M	$C_9H_{10}O_3$	4-Hydroxy-3-ethoxybenzaldehyde (Ethyl vanillin)	0
364	E	$C_9H_{10}O_4$	4-Ethoxysalicylic acid	0
365	F	$C_{16}H_{26}O_2$	4-Ethoxy-2,6-di-*tert*-butylphenol	0

LIST OF CHEMICALS TESTED—(*Cont.*)

XI. Di-, tri-, and poly-hydroxybenzenes

A. *Catechols*

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
366	K	$C_6H_6O_2$	Catechol	0
367	E	$C_{10}H_{14}O_2$	4-*tert*-Butylcatechol	70
368	E	$C_6H_2Cl_4O_2$	Tetrachlorocatechol	80
369	Sy	$C_9H_8O_4$	3,4-Dihydroxycinnamic acid (Caffeic acid)	0

B. *Resorcinols*

370	K	$C_6H_6O_2$	Resorcinol	0
371	W	$C_{12}H_{18}O_2$	4-*n*-Hexylresorcinol	100
372	I	$C_{21}H_{36}O_2$	5-Pentadecylresorcinol	0
373	I	$C_{21}H_{32}O_2$	5-Pentadecadienylresorcinol	0
374	K	$C_6H_5ClO_2$	4-Chlororesorcinol	0
375	E	$C_6H_5NO_4$	2-Nitroresorcinol	0
376	C	$C_7H_7NO_4$	2-Nitro-5-methylresorcinol (2-Nitroorcinol)	0
377	E	$C_6H_4N_2O_6$	2,4-Dinitroresorcinol	0
378	C	$C_6H_3Br_3O_2$	2,4,6-Tribromoresorcinol	20
379	K	$C_7H_6O_4$	2,4-Dihydroxybenzoic acid (β-Resorcylic acid)	0
380	E	$C_8H_8O_3$	2,4-Dihydroxyacetophenone (Resacetophenone)	0
381	E	$C_7H_7NO_3$	2,4-Dihydroxybenzaldehyde oxime (Resorcylaldoxime)	0

C. *Hydroquinones*

382	B	$C_6H_6O_2$	Hydroquinone	0
383	C	$C_9H_{10}O_2$	Allylhydroquinone	70
384	E	$C_{14}H_{22}O_2$	2,5-Di-*tert*-butylhydroquinone	100
385	C	$C_{14}H_{22}O_2$	Octylhydroquinone (1,1,3,3-Tetramethylbutyl-hydroquinone)	50
386	C	$C_{26}H_{30}O_2$	2,5-Bis(2,3,4,6-tetramethyl-phenyl) hydroquinone	0
387	C, E	$C_6H_5BrO_2$	Bromohydroquinone	0, 60, 90
388	E	$C_6H_5ClO_2$	Chlorohydroquinone	100
389	C	$C_6H_2Br_4O_2$	Tetrabromohydroquinone	0
390	E	$C_6H_2Cl_4O_2$	Tetrachlorohydroquinone	0
391	C	$C_7H_6O_4$	2,5-Dihydroxybenzoic acid (Gentisic acid)	0
392	C	$C_8H_6O_6$	2,5-Dihydroxyterephthalic acid	0
393	C	$C_8H_8O_3$	2,5-Dihydroxyacetophenone	0
394	C	$C_{12}H_{11}NO_4S$	2,5-Dihydroxybenzenesulfonanilide	0

D. *Polyhydroxybenzenes*

395	Ba	$C_6H_6O_3$	1,2,3-Trihydroxybenzene (Pyrogallol)	0
396	Me	$C_6H_6O_3$	1,3,5-Trihydroxybenzene (Phloroglucinol)	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
397	C	$C_9H_{10}O_5$	Ethyl gallate	0
398	Ed	$C_7H_8O_3$	Methyl phloroglucinol	0
399	E	$C_6H_8N_2O_4 \cdot 2HCl$	Tetrahydroxy-4-phenylenediamine dihydrochloride	0
			E. *Esters of above*	
400	E	$C_8H_8O_3$	Resorcinol monoacetate	0
401	E	$C_8H_6N_2O_7$	3,5-Dinitrohydroquinonemonoacetate	0
			XII. **Thiophenols**	
402	E	C_6H_6S	Thiophenol	60
403	E	C_7H_8S	2-Methylthiophenol	10
404	E	C_7H_8S	4-Methylthiophenol	80
405	Tr	$C_6H_3Cl_3S$	2,4,6-Trichlorothiophenol	0
			XIII. **Phenylphenols**	
			A. *2-Phenylphenols*	
406	D, E	$C_{12}H_{10}O$	2-Phenylphenol	0
407	C, E	$C_{12}H_9ClO$	2-Phenyl-4-chlorophenol	90
408	E	$C_{12}H_9ClO$	2-Phenyl-6-chlorophenol	10
409	D	$C_{12}H_8Cl_2O$	2-Phenyl-4,6-dichlorophenol	100
410	D	$C_{12}H_7Cl_3O$	2-Phenyl-x,4,6-trichlorophenol	100
411	K	$C_{12}H_9NO_3$	2-Phenyl-4-nitrophenol	100
412	K	$C_{12}H_9NO_3$	2-Phenyl-6-nitrophenol	90
413	C, K	$C_{12}H_8N_2O_5$	2-Phenyl-4,6-dinitrophenol	100
414	D	$C_{13}H_{10}O_3$	2-Phenylphenol-6-carboxylic acid (3-Phenylsalicylic acid)	0
			B. *4-Phenylphenols*	
415	D, E	$C_{12}H_{10}O$	4-Phenylphenol	0
416	E	$C_{12}H_9BrO$	4-Phenyl-2-bromophenol	50
417	D	$C_{12}H_9ClO$	4-Phenyl-2-chlorophenol	60
418	D	$C_{13}H_{10}O_3$	4-Phenylphenol-2-carboxylic acid (5-Phenylsalicylic acid)	0
			C. *Phenylcatechols and phenylhydroquinones*	
419	D	$C_{12}H_{10}O_2$	3-Phenylcatechol	0
420	D	$C_{12}H_{10}O_2$	4-Phenylcatechol	0
421	E	$C_{12}H_{10}O_2$	2-Phenylhydroquinone	10
			XIV. **Bisphenols**	
422	D, E	$C_{12}H_{10}O_2$	4,4′-Dihydroxybiphenyl	0
423	E	$C_{14}H_{14}O_2$	4,4′-Dihydroxy-3,3′-dimethylbiphenyl	60
424	C	$C_{18}H_{22}O_2$	4,4′-Dihydroxy-2,2′-dipropylbiphenyl	50
425	M	$C_{12}H_2Cl_8O_2$	x,x′-Dihydroxyoctachlorobiphenyl	100

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
426	C	$C_{28}H_{40}N_2O_2 \cdot 2HCl$	4,4′-Dihydroxy-3,3′-diallyl-5,5′-bis(diethylaminomethyl) biphenyl dihydrochloride	0
427	I	$C_{27}H_{40}O_2$	1,8- and 1,9-Bis(4′-hydroxyphenyl) pentadecane	0
			XV. Benzylphenols	
			A. 2-Benzylphenols	
428	E	$C_{13}H_{12}O$	2-Benzylphenol	80
429	Mi	$C_{14}H_{14}O$	2-Benzyl-4-methylphenol	60
430	Mi	$C_{14}H_{14}O$	2-Benzyl-6-methylphenol	30
431	M	$C_{13}H_{10}N_2O_5$	2-Benzyl-4,6-dinitrophenol	100
432	Mi	$C_{14}H_{13}BrO$	2-(2′-Bromobenzyl)-4-methylphenol	90
433	Mi	$C_{14}H_{13}BrO$	2-(3′-Bromobenzyl)-4-methylphenol	100
434	Mi	$C_{14}H_{13}BrO$	2-(4′-Bromobenzyl)-4-methylphenol	100
435	Mi	$C_{14}H_{13}ClO$	2-(2′-Chlorobenzyl)-4-methylphenol	100
436	Mi	$C_{14}H_{13}ClO$	2-(3′-Chlorobenzyl)-4-methylphenol	80
437	Mi	$C_{14}H_{13}ClO$	2-(4′-Chlorobenzyl)-4-methylphenol	100
438	Mi	$C_{14}H_{13}BrO$	2-(2′-Bromobenzyl)-6-methylphenol	100
439	Mi	$C_{14}H_{13}BrO$	2-(3′-Bromobenzyl)-6-methylphenol	50
440	Mi	$C_{14}H_{13}BrO$	2-(4′-Bromobenzyl)-6-methylphenol	60
441	Mi	$C_{14}H_{13}ClO$	2-(2′-Chlorobenzyl)-6-methylphenol	90
442	Mi	$C_{14}H_{13}ClO$	2-(3′-Chlorobenzyl)-6-methylphenol	30
443	Mi	$C_{14}H_{13}ClO$	2-(4′-Chlorobenzyl)-6-methylphenol	80
444	K	$C_{14}H_{14}O$	2-(α-Phenylethyl)-phenol	80
445	K	$C_{15}H_{16}O$	2-(α-Phenylethyl)-4-methylphenol	20
446	K	$C_{14}H_{12}N_2O_5$	2-(α-Methylbenzyl)-4,6-dinitrophenol	100
			B. 4-Benzylphenols	
447	E	$C_{13}H_{12}O$	4-Benzylphenol	0
448	M, Mi	$C_{14}H_{14}O$	4-Benzyl-2-methylphenol	50, 100
449	Mi	$C_{14}H_{13}BrO$	4-(2′-Bromobenzyl)-2-methylphenol	100
450	Mi	$C_{14}H_{13}BrO$	4-(3′-Bromobenzyl)-2-methylphenol	70
451	Mi	$C_{14}H_{13}BrO$	4-(4′-Bromobenzyl)-2-methylphenol	100

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
452	Mi	$C_{14}H_{13}ClO$	4-(2′-Chlorobenzyl)-2-methylphenol	100
453	Mi	$C_{14}H_{13}ClO$	4-(3′-Chlorobenzyl)-2-methylphenol	100
454	Mi	$C_{14}H_{13}ClO$	4-(4′-Chlorobenzyl)-2-methylphenol	100
455	K	$C_{14}H_{14}O$	4-(α-Phenylethyl)-phenol	40
456	K	$C_{14}H_{12}N_2O_5$	4-(α-Methylbenzyl)-2,6-dinitrophenol	60
457	C, E	$C_{15}H_{16}O$	4-(α,α-Dimethylbenzyl)phenol	90, 100
458	C	$C_{25}H_{20}O$	4-Triphenylmethylphenol	0
459	E, N	$C_{15}H_{14}O$	4-Hydrindylphenol	100
			XVI. **Methylenebisphenols**	
460	G	$C_{23}H_{32}O_2$	Bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl) methane	0
461	G	$C_{31}H_{48}O_2$	Bis(2-hydroxy-3-methyl-5-iso-octylphenyl) methane	0
462	D	$C_{13}H_{10}Cl_2O_2$	Bis(2-hydroxy-5-chlorophenyl) methane	100
463	Si	$C_{13}H_8Br_2Cl_2O_2$	Bis(2-hydroxy-3-bromo-5-chlorophenyl) methane	90, 100
464	Si	$C_{13}H_8Cl_6O_2$	Bis(2-hydroxy-3,5,6-trichlorophenyl) methane	100
465	D	$C_{19}H_{22}Cl_2O_2$	Bis(2-hydroxy-5-chloro-3-isopropylphenyl) methane	100
466	G	$C_{24}H_{34}O_2$	1,1-Bis(2′-hydroxy-3′-*tert*-butyl-5′-methylphenyl) ethane	0
467	G	$C_{25}H_{36}O_2$	2,2-Bis(2′-hydroxy-3′-*tert*-butyl-5′-methylphenyl) propane	0
468	C, D	$C_{16}H_{18}O_2$	2,2-Bis(4′-hydroxyphenyl) butane	70, 80
469	G	$C_{26}H_{38}O_2$	1,1-Bis(2′-hydroxy-3′-*tert*-butyl-5′-methylphenyl) isobutane	0
470	G	$C_{26}H_{38}O_2$	2,2-Bis(2′-hydroxy-3′-*tert*-butyl-5′-methylphenyl) butane	0
471	G	$C_{29}H_{36}O_2$	Bis(2-hydroxy-3-*tert*-butyl-5-methylphenyl) phenylmethane	0
472	G	$C_{28}H_{40}O_2$	1,1-Bis(2′-hydroxy-3′-*tert*-butyl-5′-methylphenyl) cyclohexane	0
473	D	$C_{15}H_{16}O_2$	2,2-Bis(4′-hydroxyphenyl) propane	20
474	D	$C_{15}H_{14}N_2O_6$	2,2-Bis(4′-hydroxy-3′-nitrophenyl) propane	90
475	M	$C_{12}H_6Cl_4O_2S$	Bis(2-hydroxy-3,5-dichlorophenyl) sulfide	100
476	C	$C_{20}H_{26}O_2S$	Bis(2-hydroxy-5-*tert*-butylphenyl) sulfide	60
477	C	$C_{12}H_{10}O_4S$	2,4′-Dihydroxydiphenylsulfone	0
478	C, E	$C_{12}H_{10}O_4S$	4,4′-Dihydroxydiphenylsulfone	0
479	E	$C_{14}H_{12}O_2$	4,4′-Dihydroxystilbene	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
480	C	$C_{13}H_{10}O_3$	2,4′-Dihydroxybenzophenone	0
481	C	$C_{13}H_{10}O_3$	4,4′-Dihydroxybenzophenone	0
482	M	$C_{14}H_8Br_2O_4$	5,5′-Dibromosalicil	90
			XVII. Phenylazophenols	
483	E	$C_{13}H_{12}N_2O$	4-Benzeneazo-2-methylphenol	50
484	E	$C_{13}H_{12}N_2O$	4-Benzeneazo-3-methylphenol	70
485	I	$C_{27}H_{40}N_2O_4S$	4-(4′-Benzeneazosulfonic acid)-3-pentadecylphenol	100
486	Co	$C_{13}H_9N_3O_5$	4-Nitrobenzeneazosalicylic acid	0
487	E	$C_{12}H_{10}N_2O_2$	4-Benzeneazoresorcinol	10
488	E	$C_{12}H_9N_3O_4$	4-(3′-Nitrobenzeneazo)-resorcinol	50
489	E	$C_{13}H_{11}N_3O_4$	4-(4′-Nitrobenzeneazo)-5-methylresorcinol	30
			XVIII. Naphthols	
			A. *1-Naphthols*	
490	Mk	$C_{10}H_8O$	1-Naphthol	0
491	M	$C_{10}H_6Cl_2O$	2,4-Dichloro-1-naphthol	50
492	E	$C_{10}H_7NO_2$	4-Nitroso-1-naphthol	100
493	E	$C_{10}H_6N_2O_5$	2,4-Dinitro-1-naphthol	100
494	A	$C_{10}H_6N_2O_8S$	2,4-Dinitro-1-naphthol-7-sulfonic acid (Naphthol Yellow S)	0
495	E	$C_{10}H_9NO$	5-Amino-1-naphthol	0
496	P	$C_{11}H_{11}NO \cdot HCl$	4-Amino-2-methyl-1-naphthol hydrochloride	0
497	E	$C_{10}H_8O_4S$	1-Naphthol-4-sulfonic acid	0
498	Du	$C_{10}H_8O_4S$	1-Naphthol-5-sulfonic acid	0
499	E	$C_{11}H_8O_3$	1-Hydroxy-2-naphthoic acid	20
500	E	$C_{17}H_{12}O_3$	Phenyl 1-hydroxy-2-naphthoate	0
501	E	$C_{11}H_7ClO_3$	1-Naphthol-4-chloro-2-carboxylic acid	80
			B. *2-Naphthols*	
502	E	$C_{10}H_8O$	2-Naphthol	0
503	C	$C_{10}H_7BrO$	6-Bromo-2-naphthol	0
504	E	$C_{10}H_5Br_3O$	1,3,6-Tribromo-2-naphthol	10
505	E	$C_{10}H_9NO \cdot HCl$	1-Amino-2-naphthol hydrochloride	90
506	E, M	$C_{10}H_7NO_2$	1-Nitroso-2-naphthol	100
507	E	$C_{10}H_7NO_8S_2$	1-Nitroso-2-naphthol-3,6-disulfonic acid	0
508	E	$C_{10}H_8O_4S$	2-Naphthol-6-sulfonic acid	0
509	Pa	$C_{10}H_8O_4S$	2-Naphthol-7-sulfonic acid	0
510	E	$C_{10}H_8O_4S$	2-Naphthol-8-sulfonic acid	0
511	Pa	$C_{10}H_8O_7S_2$	2-Naphthol-6,8-disulfonic acid	0
512	C	$C_{11}H_8O_2$	2-Hydroxy-1-naphthaldehyde	30
513	C	$C_{11}H_8O_2$	2-Hydroxy-3-naphthoic acid	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
514	C	$C_{11}H_8O_6S$	2-Naphthol-3-carboxyl-6-sulfonic acid	0
515	C	$C_{10}H_{18}O$	*cis*-Decahydro-2-naphthol	0
516	C	$C_{10}H_{18}O$	*trans*-Decahydro-2-naphthol	0
517	C	$C_{20}H_{22}NO_6$	1,2,3,4-Tetrahydro-6-hydroxy-4-(4-hydroxy-3-methoxy-phenyl)-3-hydroxymethyl-7-methoxy-2-naphthamide	0
518	C, E	$C_{20}H_{14}O_2$	1,1′-Di-2-naphthol	0, 30
519	E	$C_{16}H_{12}N_2O_4S$	Orange II	0
520	B	$C_{22}H_{16}N_4O$	Sudan III	0
521	B	$C_{24}H_{20}N_4O$	Sudan IV	0
			C. *Naphthalenediols*	
522	E	$C_{10}H_8O_2$	1,5-Dihydroxynaphthalene	0
523	C	$C_{10}H_8O_2$	2,3-Dihydroxynaphthalene	0
524	E	$C_{10}H_8O_2$	2,7-Dihydroxynaphthalene	0
525	E	$C_{10}H_8O_8S_2$	1,8-Dihydroxynaphthalene-3,6-disulfonic acid	0
526	E	$C_{16}H_{11}N_3O_{10}S_2$	1,8-Dihydroxynaphthalene-4-nitrobenzeneazo-3,6-disulfonic acid	0
			XIX. **Quinones**	
			A. *Benzoquinones*	
527	H	$C_6H_4O_2$	1,4-Benzoquinone	80
528	C	$C_6H_4O_2 \cdot C_6H_6O_2$	Quinhydrone	80
529	M	$C_6H_2Cl_2O_2$	2,5-Dichloro-1,4-benzoquinone	10
530	M	$C_6H_2Cl_2O_2$	2,6-Dichloro-1,4-benzoquinone	10
531	H	$C_6H_2F_2O_2$	2,5-Difluoro-1,4-benzoquinone	10
532	—	$C_6Br_4O_2$	Tetrabromo-1,4-benzoquinone (Bromanil)	0
533	M	$C_6Cl_4O_2$	Tetrachloro-1,4-benzoquinone (Chloranil)	40
534	M	$C_6H_2Cl_2O_4$	2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone	0
535	C	$C_8H_8O_4$	2,6-Dimethoxy-1,4-benzo-quinone	70
536	C	$C_{22}H_{36}O_2$	2,5-Dioctyl-1,4-benzoquinone	0
537	E	C_6H_4ClNO	1,4-Benzoquinonechlorimide	100
538	M	$C_6H_2Cl_3NO$	2,6-Dichloro-1,4-benzoquinone-chlorimide	30
539	E	C_6Cl_6O	Hexachlorophenol	80
			B. *1,2-Naphthoquinones*	
540	H	$C_{10}H_6O_2$	1,2-Naphthoquinone	90
541	H	$C_{10}H_5ClO_2$	3-Chloro-1,2-naphthoquinone	30
542	H	$C_{10}H_7NO_2$	4-Amino-1,2-naphthoquinone	0
543	H	$C_{10}H_6O_3$	6-Hydroxy-1,2-naphthoquinone	80
544	H	$C_{11}H_8O_2$	7-Methoxy-1,2-naphthoquinone	0
545	H	$C_{10}H_6O_5S$	1,2-Naphthoquinone-4-sulfonic acid	0

LIST OF CHEMICALS TESTED—(*Cont.*)

C. *1,4-Naphthoquinones*

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (percent)
546	H	$C_{10}H_6O_2$	1,4-Naphthoquinone	80
547	H	$C_{10}H_5BrO_2$	2-Bromo-1,4-naphthoquinone	100
548	H	$C_{10}H_5ClO_2$	6-Chloro-1,4-naphthoquinone	100
549	E	$C_{10}H_4Cl_2O_2$	2,3-Dichloro-1,4-naphthoquinone	50
550	M	$C_{10}H_5ClO_3$	2-Chloro-3-hydroxy-1,4-naphthoquinone	0
551	U	$C_{10}H_6O_5S$	1,4-Naphthoquinone-2-sulfonic acid	0
552	H, P	$C_{11}H_8O_2$	2-Methyl-1,4-naphthoquinone	100
553	C	$C_{11}H_8O_3$	2-Methyl-3-hydroxy-1,4-naphthoquinone	0
554	H	$C_{12}H_{10}O_3$	2-Methyl-3-methoxy-1,4-naphthoquinone	0
555	H	$C_{12}H_8O_4$	2-Methyl-1,4-naphthoquinone-6-carboxylic acid	0
556	H	$C_{13}H_{10}O_3$	2-Methyl-6-acetyl-1,4-naphthoquinone	100
557	H	$C_{14}H_{12}O_3$	2-Methyl-6-propionyl-1,4-naphthoquinone	100
558	H	$C_{15}H_{14}O_3$	2-Methyl-6-*n*-butyryl-1,4-naphthoquinone	90
559	H	$C_{17}H_{18}O_3$	2-Methyl-6-*n*-caproyl-1,4-naphthoquinone	90
560	H	$C_{15}H_{12}O_5$	2-Methyl-6-succinyl-1,4-naphthoquinone	0
561	H	$C_{14}H_{14}O_2S$	2-Methyl-3-*n*-propylthio-1,4-naphthoquinone	90
562	H	$C_{14}H_{14}O_2S$	2-Methyl-3-isopropylthio-1,4-naphthoquinone	90
563	H	$C_{15}H_{16}O_2S$	2-Methyl-3-*n*-butylthio-1,4-naphthoquinone	100
564	H	$C_{17}H_{18}O_3S$	2-Methyl-3-ethylthio-6-*n*-butyryl-1,4-naphthoquinone	90
565	H	$C_{14}H_{12}O_4S$	Methyl *S*-(2-methyl-1,4-naphthoquinonyl-3)-thioacetate	90
566	H	$C_{12}H_{10}O_2S$	2-Ethylthio-1,4-naphthoquinone	100
567	H	$C_{13}H_{12}O_2S$	2-Isopropylthio-1,4-naphthoquinone	100
568	H	$C_{15}H_{16}O_2S$	2-*n*-Amylthio-1,4-naphthoquinone	0
569	H	$C_{16}H_{10}O_2S$	2-Phenylthio-1,4-naphthoquinone	0
570	H	$C_{17}H_{12}O_2S$	2,2′-Tolylthio-1,4-naphthoquinone	70
571	H	$C_{16}H_{18}O_2S_2$	2,3-Bis(propylthio)-1,4-naphthoquinone	60
572	H	$C_{24}H_{18}O_2S_2$	2,3-Bis(benzylthio)-1,4-naphthoquinone	0

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (PERCENT)
573	H	$C_{15}H_{16}O_2$	2-Isoamyl-3-hydroxy-1,4-naphthoquinone	70
574	H	$C_{25}H_{36}O_2$	2-Pentadecyl-3-hydroxy-1,4-naphthoquinone	0
575	H	$C_{19}H_{15}ClO_3$	2-[3′-(*p*-Chlorophenyl)-propyl]-3-hydroxy-1,4-naphthoquinone	100
576	H	$C_{20}H_{24}O_3$	2-(4′-Cyclohexylbutyl)-3-hydroxy-1,4-naphthoquinone	100
577	H	$C_{20}H_{24}O_4$	2-(1,4-Naphthoquinonyl)-decanoic acid	0
578	H	$C_{21}H_{26}O_4$	Methyl 2-(1,4-naphthoquinonyl)-decanoate	0
579	E	$C_{18}H_{16}N_2O$	Indophenol	0
			D. *Anthraquinones*	
580	C	$C_{14}H_8O_2$	Anthraquinone	0
581	C	$C_{14}H_9NO_2$	1-Aminoanthraquinone	0
582	C	$C_{14}H_9NO_2$	2-Aminoanthraquinone	0
583	E	$C_{15}H_{11}NO_2$	1-Methylaminoanthraquinone	90
584	An	$C_{16}H_{10}O_3$	1-Acetylanthraquinone	0
585	C	$C_{15}H_{10}O_2$	2-Methylanthraquinone	0
586	C	$C_{15}H_{10}O_4$	2-Methyl-4,5-dihydroxyanthraquinone (Chrysophanic acid)	0
587	A	$C_{14}H_8O_4$	1,2-Dihydroxyanthraquinone (Alizarin)	0
588	C	$C_{14}H_8O_4$	1,4-Dihydroxyanthraquinone (Quinizarin)	0
589	C	$C_{14}H_8O_5$	1,2,4-Trihydroxyanthraquinone (Purpurin)	70
590	E	$C_{14}H_7NO_6$	1,2-Dihydroxy-3-nitroanthraquinone	40
591	E	$C_{14}H_8O_5S$	Anthraquinone-2-sulfonic acid	0
592	E	$C_{14}H_8O_8S_2$	Anthraquinone-2,7-disulfonic acid	0
593	E	$C_{14}H_7NO_7S$	1-Nitroanthraquinone-5-sulfonic acid	0
594	An	$C_{14}H_{12}O_2$	1,2,3,4-Tetrahydroanthraquinone	0
			E. *Phenanthrenequinones*	
595	C, H	$C_{14}H_8O_2$	Phenanthrenequinone	90
596	H	$C_{14}H_8O_3$	2-Hydroxyphenanthrenequinone	30
597	H	$C_{15}H_{10}O_4$	Methyl phenanthrenequinone-3-carboxylate	0
			F. *Miscellaneous*	
598	E	$C_{18}H_{10}O_2$	1,2-Benzanthraquinone	0
599	C	$C_6H_6O_4$	5-Hydroxy-2-(hydroxymethyl)-1,4-pyrone (Kojic acid)	0

LIST OF CHEMICALS TESTED—(*Cont.*)

XX. Hydroxyquinolines, -Pyrimidines, etc.

A. *Hydroxyquinolines*

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (PERCENT)
600	E	C_9H_7NO	2-Hydroxyquinoline	0
601	C	C_9H_7NO	8-Hydroxyquinoline	0
602	E	$C_{10}H_9NO$	4-Methyl-2-hydroxyquinoline	0
603	E	$C_{11}H_{11}NO$	4,8-Dimethyl-2-hydroxy-quinoline	0
604	Ca	$C_{11}H_{11}NO_2$	4-Methyl-6-methoxy-2-hydroxyquinoline	0
605	E	$C_9H_5Br_2NO$	5,7-Dibromo-8-hydroxyquinoline	0
606	M	$C_{10}H_7Cl_2NO$	5,7-Dichloro-2-methyl-8-hydroxyquinoline	10
607	M	$C_9H_5N_3O_5$	5,7-Dinitro-8-hydroxyquinoline	0
608	E	$C_9H_6INO_4S$	7-Iodo-8-hydroxyquinoline-5-sulfonic acid	0
609	C	$C_{12}H_{10}N_2O_6$	Ethyl 4-hydroxy-7-nitro-quinoline-3-carboxylate	0
610	M	$C_{16}H_{13}NO_4$	8-Hydroxyquinolinium salicylate	0

B. *Hydroxypyrimidines*

611	C	$C_4H_6N_4O \cdot H_2SO_4$	2,6-Diamino-4-hydroxypyrimidine sulfate	0
612	C	$C_4H_5N_5O_2$	2,6-Diamino-5-nitroso-4-hydroxypyrimidine	0
613	C	$C_4H_7N_5O \cdot H_2SO_4$	2,5,6-Triamino-4-hydroxypyrimidine sulfate	0
614	C	$C_4H_4N_4O_2$	6-Imino-5-isonitrosohydrouracil	0

C. *Miscellaneous*

615	P	$C_8H_5NO_3 \cdot Na$	Benzoxadione-2,4, sodium derivative	0
616	C	$C_9H_8N_2O_2$	7-Hydroxy-6-methoxy-quinoxaline	0
617	C	$C_{14}H_{10}N_2O_4$	4-Hydroxy-5-methoxyphenanthroline-3-carboxylic acid	0
618	C	$C_{11}H_{10}NO \cdot Cl$	3-Hydroxy-1-phenylpyridinium chloride	0

XXI. Metalated phenols

A. *Mercury*

619	Ed	C_6H_5ClHgO	2-Hydroxyphenylmercuric chloride	100
620	E	C_6H_5ClHgO	4-Hydroxyphenylmercuric chloride	100
621	Ed	$C_6H_4Cl_2Hg_2O$	2,4-Dichloromercuric phenol	100
622	K	C_7H_5HgNOS	4-Hydroxyphenylmercuric thiocyanate	100

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (PERCENT)
623	Ab	$C_7H_7HgNO_4$	Metaphen	100
624	Hy	$C_{20}H_{10}Br_2HgO_6$	Mercurochrome	90
			B. *Arsenic*	
625	Me	$C_{12}H_{12}As_2N_2O_2$	Arsphenamine (Salvarsan)	0
626	Me	$C_{13}H_{14}As_2N_2O_4S$	Neoarsphenamine (Neosalvarsan)	0
			XXII. **Indicators and dyes**	
627	E	$C_{20}H_{12}O_5$	Fluorescein	0
628	E	$C_{20}H_8I_4O_5$	Tetraiodofluorescein (Iodeosin)	0
629	—	$C_{20}H_{14}O_4$	Phenolphthalein	0
630	T	$C_{19}H_{14}O_5S$	Phenolsulfonphthalein (Phenol Red)	0
631	Pa	$C_{19}H_{10}N_4O_{13}S$	Tetranitrophenolsulfonphthalein	0
632	T	$C_{21}H_{18}O_5S$	*o*-Cresolsulfonphthalein (Cresol Red)	0
633	T	$C_{21}H_{14}Br_2O_5S$	Dibromo-*o*-cresolsulfonphthalein (Bromcresol Purple)	0
634	T	$C_{21}H_{14}Br_4O_5S$	Tetrabromo-*m*-cresolsulfonphthalein (Bromcresol Green)	0
635	T	$C_{27}H_{30}O_5S$	Thymolsulfonphthalein (Thymol Blue)	0
636	C	$C_{19}H_{14}O_3$	Aurin	0
637	Na	$C_{20}H_4Cl_4I_4O_5$	Rose Bengal	0
638	Hl	$C_{26}H_{20}N_4O_8S_2$	Brilliant Yellow	0
639	E	$C_{34}H_{24}N_6O_{14}S_4$	Evans Blue	0
			XXIII. **Miscellaneous**	
640	E	$C_7H_5Br_3O$	2,4,6-Tribromoanisole	0
641	C	$C_7H_3Cl_5O$	Pentachloroanisole	0
642	E	$C_{27}H_{30}O_{16}\cdot H_2O$	Rutin	0
643	C	$C_9H_{18}O$	2-Isopropylcyclohexanol	0
644	C	$C_9H_{18}O$	4-Isopropylcyclohexanol	0
645	P	$C_9H_{18}O$	*x*,*x*,*x*-Trimethylcyclohexanol	0
646	C	$C_{12}H_{16}O$	*cis*-2-Phenylcyclohexanol	0
647	C	$C_{12}H_{16}O$	*trans*-2-Phenylcyclohexanol	0
648	C	$C_{15}H_{16}O_6\cdot 2H_2O$	α-Conidendrol	0
649	C	$C_7H_9O_4P$	4,α-Dihydroxy-α-toluenephosphonic acid	0
650	Pf	$C_6H_{12}O_6$	Inositol	0
651	C	$C_{12}H_{22}O_2$	Bicyclohexyl-1,1′-diol	0
652	E	$C_9H_6O_2$	Coumarin (1,2-Benzopyrone)	0
653	C	$C_{11}H_{10}O_3$	4-Methyl-6-methoxycoumarin	0
654	C	$C_{11}H_8O_4$	3-Acetyl-4-hydroxycoumarin	0
655	E	$C_9H_5ClO_2$	6-Chlorocoumarin	0
656	—	$C_{19}H_{12}O_6$	Dicoumarol	0
657	K	$C_7H_4O_2S$	5-Hydroxy-2-ketobenzothioxole	30
658	K	$C_7H_4O_2S$	6-Hydroxy-2-ketobenzothioxole	90

LIST OF CHEMICALS TESTED—(*Cont.*)

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (PERCENT)
659	C	C_9H_7ClOS	6-Chloro-4-methyl-3-thianaphthenol	0
660	C	$C_{19}H_{18}O_2$	4-Hydroxy-2,5-dimethyl-3,4-diphenyl-2-cyclopenten-1-one	0
661	E	$C_8H_{12}O_2$	Dimethyldihydroresorcinol (Dimedon)	0
662	E	$C_{14}H_{12}O_2$	Benzoin	0
663	C	$C_{12}H_{14}O_2$	2,2,4-Trimethyl-1,2H-benzopyran-6-ol	0
664	C	$C_{15}H_{10}O_3$	3-Hydroxy-2-anthroic acid	0
			XXIV. Metal salts of phenols	
			A. *Copper salt of*:	
665	Tr	C_6H_6O	Phenol	100
666	Tr	$C_9H_{12}O$	2,3,5-Trimethylphenol	100
667	Tr	C_6H_5ClO	2-Chlorophenol	90, 100
668	Tr	$C_6H_4Br_2O$	2,4-Dibromophenol	40, 80
669	Tr	$C_6H_4Cl_2O$	2,4-Dichlorophenol	60, 80
670	Tr	$C_6H_3Br_3O$	2,4,6-Tribromophenol	80, 90, 100
671	Tr	$C_6H_3Cl_3O$	2,4,5-Trichlorophenol	100
672	Tr	$C_6H_2Cl_3O$	2,4,6-Trichlorophenol	90, 100
673	Tr	$C_6H_3I_3O$	2,4,6-Triiodophenol	100
674	Tr	$C_6H_2Cl_4O$	2,3,4,6-Tetrachlorophenol	100
675	Tr	C_6HBr_5O	Pentabromophenol	30, 100
676	M	C_6HCl_5O	Pentachlorophenol	100
677	Tr	$C_6H_8N_2O$	2,4-Diaminophenol	0, 80
678	Tr	C_8H_9ClO	4-Chloro-3,5-dimethylphenol	100
679	Tr	$C_{10}H_{13}ClO$	4-Chloro-2-isopropyl-5-methylphenol	100
680	Tr	$C_7H_6Br_2O$	2,4-Dibromo-6-methylphenol	60, 80, 100
681	Tr	$C_7H_6Cl_2O$	2,4-Dichloro-5-methylphenol	100
682	Tr	$C_8H_7Cl_3O$	2,4,6-Trichloro-3,5-dimethylphenol	100
683	Tr	$C_7H_4Cl_4O$	2,3,4,6-Tetrachloro-5-methylphenol	90, 100
684	Tr	$C_8H_8O_3$	Methyl 2-hydroxybenzoate	100
685	Tr	$C_8H_8O_3$	Methyl 4-hydroxybenzoate	90
686	Tr	$C_{10}H_{12}O_3$	*n*-Propyl 4-hydroxybenzoate	100
687	Tr	$C_7H_8O_2$	4-Methoxyphenol	100
688	Tr	$C_6H_2Cl_4O_2$	Tetrachlorohydroquinone	10, 80, 100
689	D	$C_{13}H_{10}O_3$	2-Phenylphenol-6-carboxylic acid* (Copper 3-phenylsalicylate)	100
690	Tr	$C_{12}H_2Cl_8O_2$	*x,x'*-Dihydroxyoctachlorobiphenyl	100
691	Tr	$C_{10}H_6Cl_2O$	2,4-Dichloro-1-naphthol	90
692	Tr	$C_{10}H_8O$	2-Naphthol	100
693	M	C_9H_7NO	8-Hydroxyquinoline	90, 100
694	Tr	$C_8H_{12}O_2$	Dimethyldihydroresorcinol	100

* In this compound, the copper is attached to the carboxyl group.

LIST OF CHEMICALS TESTED—(*Cont.*)

B. *Other salts of pentachlorophenol*

NO.	SUPPLIER	FORMULA	CHEMICAL COMPOUND	MORTALITY (PERCENT)
695	Tr		Barium salt	100
696	Tr		Cadmium salt	100
697	Tr		Cobalt salt	100
698	Tr		Lead salt	100
699	Tr		Mercuric salt	100
700	C, Tr		Nickel salt	100
701	M		Zinc salt	100

LIMITATIONS OF THE EXPERIMENTS

It was realized from the start of the screening program that there were certain limitations inherent in the results obtained from the use of 10 snails for a test and 10 ppm of test chemical. Duplicate tests have demonstrated time and time again that this technique will distinguish without fail between effective and ineffective compounds. However, it does not reveal gradations in effectiveness which may well exist between compounds giving in our tests a 100 per cent mortality; nor does it reveal the "degree of ineffectiveness" of a compound.

To test the "degree of lethality" most of the compounds which gave 100 or 90 per cent lethality in preliminary screening were tested at lower concentrations, namely, 5, 3, and 1 ppm. The results of the tests are shown in Table 1.

The "degree of ineffectiveness" might appear to be only of academic interest. It does, however, explain the results obtained with a number of compounds. For instance, phenol (No. 1) gave 0 per cent mortality, as did 4-chlorophenol (No. 71). 2-Isopropylphenol (No. 16) gave 0 per cent, whereas 4-chloro-2-isopropylphenol (No. 141) gave 80 per cent. It would appear that phenol is of such a low order of inactivity that the addition of a halogen does not increase the activity sufficiently to raise it above the "ineffective" level of our tests. On the other hand, 2-isopropylphenol, although ineffective by our standards, may be closer to the borderline of effectiveness than phenol, and the addition of a halogen to it in the 4-position raises it into the effective range. Other examples could be cited.

Many of the compounds which showed high activity at 10 ppm fell off rapidly at lower concentrations and probably are of doubtful value as potential molluscicides. On the other hand certain compounds were very active at 5, 3, and occasionally 1 ppm. In general, the compounds which retained their activity at lower concentrations were the highly halogenated phenols (Nos. 79, 83, 87, 88, and 90), the dinitroalkyl- and arylphenols (Nos. 237, 238, 239, 240, 243, 244, 413, 431, and 446), the "bisphenol" type of compound (Nos. 462, 463, 464, 465, 475, and 482), and the mercurated phenols (Nos. 619, 620, 621, 622, and 623).

The molluscicidal potentialities of some of the compounds listed in Table 1, for instance, 2,4,6-tribromophenol, 2,4,5-trichlorophenol, pentachlorophenol, and 2,4-dinitro-6-cyclohexylphenol and its dicyclohexylamine salt have been demonstrated in field trials (2, 5, 6, 8, 10, 11). On the basis of availability and cost, pentachlorophenol or its sodium salt would appear to be the molluscicide

TABLE 1

Results obtained at lower concentrations with certain phenolic type compounds 100 or 90 per cent lethal at 10 ppm

COMPOUND NUMBER	COMPOUND	MORTALITY OF *Australorbis glabratus* (PER CENT) AT:			
		10 ppm	5 ppm	3 ppm	1 ppm
31	4-*tert*-Octylphenol	100	60	0	0
32	2,4-Di-*tert*-butyl-5-methylphenol	90	10	0	0
78	2,5-Dibromophenol	100	70	20	0
79	2,6-Dibromophenol	100	100	80	0
83	2,4,6-Tribromophenol	100	100	100	0
85	2,4,5-Trichlorophenol	100	60	50	0
86	2,4,6-Trichlorophenol	100	50	0	0
87	2,4,6-Triiodophenol	100	100	100	10
88	2,3,4,6-Tetrachlorophenol	100	100	90	0
89	Pentabromophenol	100	100	50	0
90	Pentachlorophenol	100	100	100	10
91	4-Bromo-2,6-dichlorophenol	90	70	0	0
92	2,6-Dibromo-4-chlorophenol	100	40	10	0
93	4-Chloro-2,6-diiodophenol	100	70	40	0
94	3-Bromo-2,4,5,6-tetrachlorophenol	100	50	20	0
95	3,5-Dibromo-2,4,6-trichlorophenol	100	80	70	10
115	4-Methylaminophenol sulfate	100	60	0	0
118	4-Dimethylaminophenol sulfate	90	10	10	0
157	2-Chloro-4-octylphenol	100	50	10	0
164	2-Chloro-4-cyclohexylphenol	90	90	30	0
166	4-Chloro-2-cyclohexylphenol	100	80	40	10
170	2-Bromo-4-*tert*-butylphenol	90	50	0	0
181	4-Thiocyano-2-amylphenol	100	100	0	0
182	4-Thiocyano-2-*tert*-butyl-5-methylphenol	100	20	0	0
204	2,6-Dichloro-4-*tert*-amylphenol	100	50	30	0
207	2,6-Dichloro-4-octylphenol	100	80	50	0
214	2,4,6-Trichloro-3,5-dimethylphenol	90	0	0	0
215	2,3,4,5-Tetrabromo-6-methylphenol	100	90	20	0
216	2,3,4,6-Tetrachloro-5-methylphenol	100	40	30	0
235	2,4-Dinitro-3-methyl-5-ethylphenol	100	10	20	0
236	2,4-Dinitro-6-isopropylphenol	100	0	0	0
237	2,4-Dinitro-3-methyl-6-isopropylphenol	100	100	80	0
238	2,4-Dinitro-6-*sec*-butylphenol	100	100	100	90[a]
239	2,4-Dinitro-3-methyl-6-*tert*-butylphenol	100	100	100	30
240	2,4-Dinitro-6-*sec*-amylphenol	100	100	90	50
243	2,4-Dinitro-6-cyclohexylphenol	100	100	100	80[a]
244	2,4-Dinitro-6-cyclohexylphenol, dicyclohexylamine salt	100	100	100	70[a]
250	2,6-Dinitro-4-*sec*-amylphenol	100	60	10	0
252	2,6-Dinitro-4-cyclohexylphenol	90	100	40	0
270	2,6-Dibromo-4-nitrophenol	100	0	0	0
271	2,6-Dichloro-4-nitrophenol	100	30	0	0
371	4-*n*-Hexylresorcinol	100	20	0	0
384	2,5-Di-*tert*-butylhydroquinone	100	10	10	0
388	Chlorohydroquinone	100	90	90	20
407	2-Phenyl-4-chlorophenol	90	50	0	0
409	2-Phenyl-4,6-dichlorophenol	100	0	0	0

TABLE 1—(*Cont.*)

COMPOUND NUMBER	COMPOUND	MORTALITY OF *Australorbis glabratus* (PER CENT) AT:			
		10 ppm	5 ppm	3 ppm	1 ppm
410	2-Phenyl-*x*,4,6-trichlorophenol	100	10	0	0
411	2-Phenyl-4-nitrophenol	100	80	70	0
412	2-Phenyl-6-nitrophenol	90	30	0	0
413	2-Phenyl-4,6-dinitrophenol	100	100	100	60
425	*x*,*x*′-Dihydroxyoctachlorobiphenyl	100	50	30	0
431	2-Benzyl-4,6-dinitrophenol	100	100	100	40
432	2-(2′-Bromobenzyl)-4-methylphenol	90	0	10	0
433	2-(3′-Bromobenzyl)-4-methylphenol	100	0	10	0
434	2-(4′-Bromobenzyl)-4-methylphenol	100	50	0	0
435	2-(2′-Chlorobenzyl)-4-methylphenol	100	30	10	0
437	2-(4′-Chlorobenzyl)-4-methylphenol	100	70	10	0
438	2-(2′-Bromobenzyl)-6-methylphenol	100	90	0	0
441	2-(2′-Chlorobenzyl)-6-methylphenol	90	60	10	0
446	2-(α-Methylbenzyl)-4,6-dinitrophenol	100	100	90	0
448	4-Benzyl-2-methylphenol	100	0	0	0
449	4-(2′-Bromobenzyl)-2-methylphenol	100	70	0	0
451	4-(4′-Bromobenzyl)-2-methylphenol	100	70	0	0
452	4-(2′-Chlorobenzyl)-2-methylphenol	100	40	0	0
453	4-(3′-Chlorobenzyl)-2-methylphenol	100	10	0	0
454	4-(4′-Chlorobenzyl)-2-methylphenol	100	70	30	0
457	4-(α,α-Dimethylbenzyl)phenol	100	0	0	0
459	4-Hydrindylphenol	100	0	0	0
462	Bis(2-hydroxy-5-chlorophenyl) methane	100	100	80	20
463	Bis(2-hydroxy-3-bromo-5-chlorophenyl) methane	100	100	80	10
464	Bis(2-hydroxy-3,5,6-trichlorophenyl) methane	100	100	100	90[b]
465	Bis(2-hydroxy-5-chloro-3-isopropylphenyl) methane	100	90	90	90
474	2,2-Bis(4′-hydroxy-3′-nitrophenyl) propane	90	0	0	0
475	Bis(2-hydroxy-3,5-dichlorophenyl) sulfide	100	100	100	0
482	5,5′-Dibromosalicil	90	90	90	10
485	4-(4′-Benzeneazosulfonic acid)-3-pentadecylphenol	100	90	10	0
492	4-Nitroso-1-naphthol	100	90	30	0
493	2,4-Dinitro-1-naphthol	100	10	0	0
506	1-Nitroso-2-naphthol	100	60	40	0
537	1,4-Benzoquinonechlorimide	100	90	80	0
619	2-Hydroxyphenylmercuric chloride	100	100	100	60
620	4-Hydroxyphenylmercuric chloride	100	100	100	60
621	2,4-Dichloromercuric phenol	100	100	100	40
622	4-Hydroxyphenylmercuric thiocyanate	100	100	100	60
623	Metaphen	100	100	90	0
624	Mercurochrome	90	0	0	0
658	6-Hydroxy-2-ketobenzothioxole	90	0	0	0

[a] Results at 2 ppm.

[b] 0.5 ppm: 80 per cent mortality; 0.3 ppm: 50 per cent mortality; 0.1 ppm: 0 per cent mortality.

of choice at this time. Disregarding economic and production factors, a number of the compounds would certainly appear to be valuable for field use. The further problem of specificity for snails versus economically valuable aquatic life has not been studied in this particular project.

DISCUSSION

A sufficient number of phenols have been tested to make possible the drawing of many conclusions concerning the correlation of molluscicidal activity and chemical structure. Some of these are pointed out below.

There appears to be no correlation of activity with molecular weight, acidic strength of the phenol, percentage composition, etc. There appears to be a correlation between phenol coefficient (bactericidal property) and molluscicidal activity. Only future work will show whether the lethal action exerted by the phenols involves the same mechanism or mechanisms (metabolic or other) in snails as in bacteria. There also appears to be some relationship between the fungicidal and molluscicidal activities of phenols.[7]

The lower alkylated phenols are ineffective as molluscicides. Alkyl groups in the 2- and 4- positions are synergistic. The presence of these groups in both the 2- and 6- positions greatly reduces the activity of otherwise effective compounds. Activity seems to reach a peak at about 8 carbons in the alkyl group and diminishes with the addition of more carbons. This bears a remarkable resemblance to the bactericidal or bacteriostatic action of phenols, perhaps best illustrated by the high activity of hexylresorcinol (No. 371). Cyclohexyl is an effective group, see 2-cyclohexylphenol (No. 49) and 4-cyclohexylphenol (No. 50). A group in the 2-position is somewhat more effective than the same group in the 4-position. Groups in the 3-position contribute very little to activity; sometimes they actually decrease activity.

Although the lower halogenated phenols are ineffective, the higher ones are among the most effective molluscicides. The dividing line for activity is at 2 bromines and at 3 chlorines. Bromine seems to be somewhat more effective than chlorine. An insufficient number of iodinated and fluorinated phenols have been tested to make possible any conclusion regarding them. Halogens in the 3-position appear to be less effective than halogens in the 2-, 4-, or 6- position. The thiocyano group appears to be very effective; compare Nos. 68, 71, 74, and 76.

The weight of the halogen part of the molecule does not appear to determine the activity. For example, 2-iodophenol (I = 127) is ineffective, whereas 2,4,5- and 2,4,6-trichlorophenols (3 Cl = 106) are 100 per cent effective. It would be interesting to test tri-, tetra-, and pentafluorophenols. However, to our knowledge none of these has been synthesized to date.

Nitro groups alone are ineffective, although, as pointed out below, they enhance the activity of other groups.

The aminophenols are extremely interesting. 2- and 3-Aminophenol are ineffective. 4-Aminophenolhydrochloride had 30 per cent activity, whereas 4-

[7] Based on fungicidal data provided us by Dr. Harold D. Shirk, National Research Council.

aminophenol itself was 70 and 100 per cent active in 2 separate tests. The 4-aminophenol was an old sample which had acquired considerable color, suggesting transformation to benzoquinonechlorimide or benzoquinone. The high activity of the latter (Nos. 537 and 527, respectively) would tend to support this hypothesis.

N-Substituted 4-aminophenols are active. Insufficient 2- and 3-substituted compounds have been tested to make comparisons possible.

There is sometimes reinforcement of the activity of 2-alkylphenols by the presence of a halogen or a thiocyano group in the 4-position (see later discussion). However, if the halogen is in the 6-position, activity disappears (Table 2).

In the case of the 4-alkylphenols, there is sometimes reinforcement of the activity by the presence of a halogen in the 2-position. Halogens in both the 2- and 6-positions usually increase the activity of the 4-alkylphenols (Table 3).

TABLE 2

Influence of halogen and thiocyano groups on the activity of 2-alkylphenols

R				
	Snail mortality, per cent			
H	0	0	0	40
CH_3	0	0	0	0, 30
C_2H_5	0	—	—	70, 80
C_3H_7	0	80	0	80
C_4H_9	—	—	—	—
C_5H_{11}	10	60	0	90, 100
C_6H_{11}*	80	100	0	—

* Cyclohexyl.

Although nitro groups alone, as mentioned above, are ineffective, they exert a powerful reinforcing effect on alkyl groups. Some of the nitro-alkylphenols are among the most effective compounds (Table 4). Especially active are the 2,4-dinitro-6-alkylphenols.

The phenolic aldehydes, ketones, and their derivatives are characteristically ineffective. The introduction of a carbonyl group into an otherwise active compound lowers the activity appreciably.

Carboxyl, sulfonic, and arsonic acid groups likewise lower activity considerably. No really active compounds of this class are to be found among the 48 tested.

The alkoxyphenols are, as a group, ineffective. Compounds which would otherwise be effective suffer a reduction in activity when alkoxy groups are introduced.

The introduction of a second hydroxyl group into a phenol leads to unpredictable activity (Table 5).

The number of thiophenols tested is insufficient to draw any conclusions regarding their activity.

TABLE 3

Influence of halogens on the activity of 4-alkylphenols

R					
	Snail mortality, per cent				
H	0	0	0	0	100
CH_3	0	0	—	0	0
C_2H_5	0	10	—	20	—
C_3H_7	0	0	—	60	—
C_4H_9	0	10	90	80	70
C_5H_{11}	0	30	—	100	—
C_6H_{11}*	70	90	—	70	—
C_8H_{17}	100	100	—	100	—
C_9H_{19}	40, 70	10	—	40	—

* Cyclohexyl.

TABLE 4

Influence of nitro groups on the activity of alkylphenols

R					
	Snail mortality, per cent				
H	0	0	0	0	0
CH_3	0	0	0	50	—
C_2H_5	—	0	—	—	0
C_3H_7	0	20	—	100	20
C_4H_9	—	—	—	100	40
C_5H_{11}	10	—	—	100	50[a], 100[b]
C_6H_{11}*	80	—	—	100	90
$C_{15}H_{31}$	—	0	—	0	—

* Cyclohexyl.
[a] *Tert*-amyl.
[b] *Sec*-amyl.

Although 2- and 4-phenylphenol themselves are ineffective, the presence of halogens or nitro groups on the phenol ring leads to activity in these compounds (Table 6).

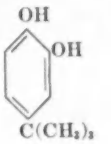
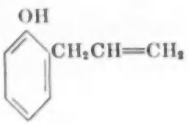
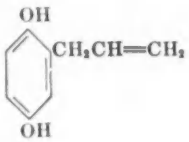
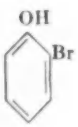
The 2-benzylphenols are characterized by their high activity. Every one of the 17 compounds in this class showed some activity. The 4-benzylphenols are

also quite effective, being comparable to the 2-benzylphenols. These compounds warrant further investigation.

In general, the benzoquinones and naphthoquinones are quite effective. Activity decreases with increased substitution. Halogens have a peculiarly deleterious effect upon activity (Table 7). Anthraquinones are generally of a low

TABLE 5

Influence of a second hydroxyl group on the activity of phenols

	0%		70%
	0%		70%
	0%		60, 90%
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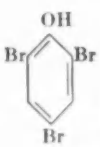
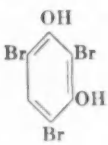
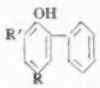
	100%		20%

order of activity. The relation between phenolic type compounds and the quinone type compounds is interesting. It may be worth while considering whether or not the activity of phenols as molluscicides is due to their conversion within the body of the snail to quinone type substances with the toxic effect due to the latter. The generally high order of activity of the quinones might point in this direction.

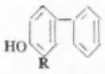
Six mercurated phenols were tested. All were very active. The activity is undoubtedly due to the presence of the mercury, because every mercury compound

TABLE 6

Influence of halogens or nitro groups on the activity of phenylphenols



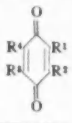
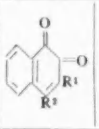
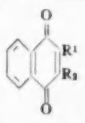
R	R′	Snail mortality, per cent
H	H	0
Cl	H	90
H	Cl	10
Cl	Cl	100
NO_2	H	100
H	NO_2	90
NO_2	NO_2	100



R	Snail mortality, per cent
H	0
Br	50
Cl	60

TABLE 7

Influence of halogens on the activity of benzoquinones and naphthoquinones

R^1	R^2	R^3	R^4		R^1	R^3		
				Snail mortality, per cent				
H	H	H	H	80	H	H	90	80
Cl	H	Cl	H	10	Br	H	—	100
Cl	H	H	Cl	10	Cl	Cl	—	50
F	H	F	H	10	Cl	OH	—	0
Br	Br	Br	Br	0	Cl	H	30	—
Cl	Cl	Cl	Cl	40				
Cl	OH	Cl	OH	0				

tested to date in this laboratory has been active. Indeed, mercury compounds are among the most active molluscicides so far discovered.[8]

[8] After our manuscript had been completed, there came to our attention a paper by Szumlewicz & Kemp (1951, *Rev. bras. Malariol. e Doenças Trop.*, v. 3, pp. 389–406 (in Portuguese), 407–422 (in English)), pointing out the efficacy of phenylmercuric acetate as a molluscicide.

Metallic salts.—Phenols form salts with metallic ions. Copper salts are known to be extremely toxic to snails. It seemed logical to prepare and test copper salts of phenolic type compounds, thereby combining in a single molecule the effect of 2 entirely different molluscicidal agents. The testing of these copper salts was especially difficult due to their extremely low solubilities and erratic results often were obtained. In general, they were highly effective. The copper salts of ineffective phenols were very active.

Finally, a number of metallic salts of pentachlorophenol were prepared and tested. All were 100 per cent effective at 10 ppm. The results with these compounds at 5, 3, and 1 ppm paralleled those obtained with pentachlorophenol itself (Table 8).

TABLE 8

Activity of metallic salts of pentachlorophenol

COMPOUND NUMBER		MORTALITY OF *Australorbis glabratus* (PER CENT) AT:			
		10 ppm	5 ppm	3 ppm	1 ppm
90	Pentachlorophenol	100	100	100	10
695	Pentachlorophenol, barium salt	100	90	80	10
696	Pentachlorophenol, cadmium salt	100	90	30	0
697	Pentachlorophenol, cobalt salt	100	90	70	0
676	Pentachlorophenol, copper salt	100	100	100	90
698	Pentachlorophenol, lead salt	100	60	20	10
699	Pentachlorophenol, mercuric salt	100	100	90	20
700	Pentachlorophenol, nickel salt	100	100	60	30
701	Pentachlorophenol, zinc salt	100	100	90	10

DISTRIBUTION ACCORDING TO ACTIVITY

The original choice of 10 ppm as the test concentration was arbitrary. It proved to be most fortunate, however, because it readily distinguished between the very effective and the very ineffective compounds, with the minority of the compounds having intermediate activity. A summary of the activities of all of the phenolic type compounds is shown in Table 9. As stated earlier, the groupings are for convenience only, and there is much overlapping in the various groups.

SUMMARY

Seven hundred and one phenolic type compounds were tested for their activity against *Australorbis glabratus*, the snail vector of *Schistosoma mansoni* in the Western Hemisphere.

Correlations have been drawn between structure of the chemical compounds and molluscicidal activity.

The most active compounds were found among the highly halogenated phenols, the dinitroalkyl- and arylphenols, the "bisphenol" type of compound and the mercurated phenols.

On the basis of availability and cost, pentachlorophenol or its salts would appear to be the molluscicide of choice at this time.

TABLE 9

Distribution of phenolic type compounds according to molluscicidal activity

CHEMICAL GROUP	TOTAL NUMBER	COMPOUNDS IN GROUP ARRANGED ACCORDING TO MOLLUSCICIDAL ACTIVITY (% OF SNAILS KILLED BY 24-HOUR EXPOSURE TO 10 PPM)						
		0–10 %	20–30 %	40–50 %	60–70 %	80 %	90 %	100 %
Alkylated phenols	64	50	2	2	3	3	1	3
Halogenated phenols	30	13	0	2	1	0	1	13
Nitro-, amino- and nitroso-phenols	31	20	3	0	4	0	2[a]	2
Alkylated-halogenated phenols	90	51	7	5	8	8	3	8
Alkyl-nitro- and alkyl-amino-phenols	50	28	4	6	2	0	1	9
Halogenated-nitrophenols	8	4	0	0	1	1	0	2
Phenolic aldehydes, ketones and derivatives	13	11	1	0	1	0	0	0
Hydroxyaromatic acids, and their esters and amides	48	47	0	0	1	0	0	0
Alkoxyphenols	30	29	1	0	0	0	0	0
Di-, tri-, and poly-hydroxybenzenes	36	27	1	1	2	1	1	3
Thiophenols	4	2	0	0	1	1	0	0
Phenylphenols	16	8	0	1	1	0	2	4
Bisphenols	6	3	0	1	1	0	0	1
Benzylphenols	32	2	3	2	4	4	2	15
Methylenebisphenols	23	13	1	0	1	1	2	5
Phenylazophenols	7	2	1	2	1	0	0	1
Naphthols	37	28	3	1	0	1	1	3
Quinones	73	37	3	3	5	5	9	11
Hydroxyquinolines, -pyrimidines, etc.	19	19	0	0	0	0	0	0
Metalated phenols	8	2	0	0	0	0	1	5
Indicators and dyes	13	13	0	0	0	0	0	0
Miscellaneous	25	23	1	0	0	0	1	0
Total compounds	663	432	31	26	37	25	27	85
Phenol	1	1						
Copper and other metal salts of phenols	37	0	0	0	0	3	2	32
Grand Total	701	433	31	26	37	28	29	117

[a] 1 compound, No. 125, 48-hr. exposure.

ACKNOWLEDGMENT

Grateful acknowledgment for technical assistance is made to Richard L. Dorman, G. Terry Gilleland, and Elizabeth M. Landry.

The authors are deeply indebted to all who contributed chemical compounds for the tests, and particularly wish to extend appreciation for generous cooperation to: Carbide and Carbon Chemical Company, Chemical-Biological Coordination Center (and all suppliers of the chemicals to that organization), Dow Chemical Company, Edwal Laboratories, Inc., Firestone Tire and Rubber Company, Fisher Scientific Company, Gulf Research and Development Company, Irvington Varnish and Insulator Company, Koppers Company, Inc., Monsanto Chemical Company, Parke, Davis and Company, Sharples Chemical Company, Sindar Corporation, and to Drs. W. R. Boehme, E. Bueding, L. F. Fieser, G. C. Finger, and R. C. Huston.

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12. Nolan, M. O. and Berry, E. G. 1949. Preliminary field trials with laboratory-tested molluscacides, *Pub. Health Rep.* **64:** 942–949.

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgment must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

Hypersplenism and Surgery of the Spleen, by William Dameshek, M.D., and C. Stuart Welch, M.D., Pratt Diagnostic Hospital, New England Center Hospital and Tufts College Medical School, Boston, Massachusetts. Based on exhibits presented at the Annual Meetings in 1947 and 1951 of the American Medical Association. 84 pp. with many illustrations, some in color. New York: Grune & Stratton, Inc., 1953. $10.00.

Survey of Antimalarial Agents. Chemotherapy of *Plasmodium gallinaceum* infections; toxicity; correlation of structure and action, by G. Robert Coatney, W. Clark Cooper, Nathan B. Eddy and Joseph Greenberg. 323 pp. Public Health Monograph No. 9; Washington, D. C., Public Health Service Publication 193, Government Printing Office, Superintendent of Documents. $1.25.

Manual de Micologia Médica, by Carlos Da Silva Lacaz, Professor de Microbiologia e Imunologia da Faculdade de Medicina de Sorocaba. 1953 edition. 370 pp. São Paulo, Organização "Liteci", Caixa Postal, 7395.

Modern Concepts of Leprosy, by Harry L. Arnold, Jr., M.D., F.A.C.P., Department of Dermatology, Straub Clinic, Honolulu, Hawaii. Publication No. 145, American Lecture Series. 105 pp. with 33 illustrations. Springfield, Illinois: Charles C. Thomas. 1953. Price $3.75. Published simultaneously by Blackwell Scientific Publications, Ltd., Oxford, England and The Ryerson Press, Toronto, Canada.

Dermatology in General Practice, by Jacob Hyams Swartz, M.D., Assistant Professor of Dermatology, Harvard Medical School; Consulting Dermatologist, Massachusetts General Hospital; with a foreword by C. Guy Lane, M.D., Professor Emeritus, Department of Dermatology, Harvard Medical School. 581 pp. with 253 illustrations. Baltimore: The Williams & Wilkins Co., 1953. Price $11.00.

First Report of the Expert Committee on Influenza. WHO Technical Report Series No. 64. Available also in a French edition. 32 pp. Geneva: W. H. O., Palais des Nations, or Columbia University Press, International Documents Services, 2960 Broadway, New York 27, N. Y. Price 1/6, $0.20; Sw. fr. 0.80; Fr. fr. 65.

Modern Concepts of Communicable Disease, by Morris Greenberg, M.D., M.S.P.H., Director, Bureau of Preventable Diseases, New York City Department of Health; Assistant Professor of Epidemiology, Columbia University, and Anna V. Matz, R.N., M.A., Public Health Nursing Consultant in Communicable Diseases, New York City Department of Health. Foreword by Harry S. Mustard, M.D. 567 pp. with illustrations. New York: G. P. Putnam's Sons, 1953. $6.20.

BOOK REVIEWS

Handbook of Tropical Dermatology and Medical Mycology. Vol. 1., Ed. by R. D. G. Ph. Simons, Senior Lecturer at Dermatological Clinic of the University of Leyden and Dermatologist in charge at the Civilian Hospital, Amsterdam, with 40 international collaborators. 845 pp. 587 illustrations. Amsterdam, Houston, New York, London, Elsevier Publishing Co. 1952. Price $15.00.

This book is an excellent comprehensive collaboration of some 40 competent investigators into almost as many aspects of Tropical Dermatology. Each chapter is a brief and yet clearly presented definite survey, from personal experience often by a person of world

renown in his particular field. Though each chapter deserves careful note, special attention is directed to the following sections:

"Pigmentation" (by Becker, Siemen, Marchionini); "Syphilis" (by Eliasson and Spencer); "Bejel" (by Hudson); "Yaws" (by Jackett); "Pinta" (by Pardo-Castello and Costenado); "Leprosy" (by Simons, Arnold, Muir and Dharmendra, Schipman, Campos, Molina—articles by the aforementioned); "T.B. and Sarcoid" (by Fasal); "Rickettsiae" (by Keddie); "Cutaneous Leishmaniasis" (by Katzenellenbogen).

Commendation is due the editor Simons for the uniform excellence of the translation. His touch is apparent throughout his Handbook. The tone and fluency of his introductory chapter "General Survey" has the pervasiveness needed to keep the book from becoming a conflicting mixture that all too many encyclopaedic works are. The excellent illustrations and the extensive bibliography render this "Handbook" especially valuable as a reference for dermatologists, general practitioners and medical students interested in tropical medicine and for hospitals and general medical libraries.

I do not agree entirely with some of the statements made by a few of the contributors and there are a small number of errors in various chapters. I have no hesitancy in recommending this "Handbook" most highly.

CHARLES R. REIN

BCG Vaccination. Studies by the WHO Tuberculosis Research Office, Copenhagen, Monograph Series No. 12. By Lydia B. Edwards, Chief of Field Studies, Carroll E. Palmer, Director, and Knut Magnus, Assistant Statistician. 307 pp. Geneva, World Health Organization, Palais des Nations. 1953. Price: 15/-; $3.00; Sw. fr. 12.-

This three-hundred page monograph provides the first coordinated account of an elaborate investigation of BCG vaccination organized by the Tuberculosis Research Office of the World Health Organization and conducted in cooperation with the International Tuberculosis Campaign and Statens Seruminstitut of Copenhagen. It sets forth the results of three years of intensive study of a number of crucial problems which must be solved before a satisfactory estimate of the value of BCG vaccination is possible. The volume deals largely with the development of tuberculin allergy after BCG vaccination, a phenomenon in which the character of the vaccine and the manner of its administration are highly important. Response to tuberculin is recognized by the authors as the only guide presently available on the effectiveness of the vaccinating procedure. Chapters are included on the effects of light, heat and storage on the potency of the vaccine as measured by development of allergy, and on the relative roles of dead and live bacilli in various preparations of the vaccine in eliciting allergy. The monograph is extraordinarily well documented in approximately 150 pages of appendices, in which the results of all the tests are tabulated. A general summary of four pages affords a ready review of the major facts learned.

The records submitted are based on a study of tuberculin tests and BCG vaccination in a group of 40,000 school children in Denmark, Mexico, Egypt and India, of whom 23,000 received the vaccine. Post-vaccination examinations were made 2-3 months, one year and two years after the immunizing procedure. Statistically valid samples furnishing suitable controls were set up for a wide variety of comparisons. For the purposes of the study some three hundred groups were established.

The data set forth clearly indicate numerous pitfalls inherent in determining the value of BCG vaccination, and complex factors which must be taken into account in any acceptable analysis. The book does not attempt an evaluation of the vaccine in protection against tuberculosis. That much desired and long awaited estimate is deferred pending the accumulation of adequate data.

ESMOND R. LONG

Atlas of Medical Mycology. by Emma Sadler Moss, Clinical Professor of Pathology, and Albert Louis McQuown, Clinical Assistant Professor of Pathology, Louisiana State

University School of Medicine. 245 pp. 248 illustrations. Baltimore, Maryland, The Williams & Wilkins Company, 1953. Price $8.00.

Fungus infections simulate malignancy and a wide variety of diseases caused by other infectious agents. A definitive diagnosis of a fungus infection depends, therefore, on the detection, isolation and identification of the etiologic agent. The personnel of the laboratory, clinical or pathological, are responsible for this service. Moss and McQuown present their "Atlas of Medical Mycology" to lighten the task of the personnel in such laboratories.

In this Atlas, a brief chapter on classification is followed by chapters on the various diseases caused by fungi; each chapter is supplemented with clinical and mycological illustrations which total 248 figures. The text also includes 10 charts which summarize clinical and mycological information in tabular form. Chapters on methods, immunology, contaminants, culture media, a glossary and formulary complete the book. Eighteen references are found on the last page.

The clinical illustrations in this book are excellent. The mycological illustrations, however, often fail to demonstrate the characteristic microscopic morphology of the fungi necessary for their identification. This lack of essential microscopic detail for specific identification cannot be overcome by the appearance of a colony on any medium, the size of a colony at any age or by the fluorescence of a colony under the Wood's light. In this respect, Charts 8 & 9 are of no diagnostic value.

Errors occasionally appear in the text concerning methods for identification. In the Chapter on Moniliasis, for example, corn meal agar cultures of *Candida albicans* should be examined for chlamydospores in 24–48 hours; Sabouraud's glucose broth tube cultures should be examined for surface growth in 48 hours; bubbly froth is produced on the surface of Sabouraud's glucose broth tubes by *C. tropicalis* not *C. albicans*; and AG is produced in sucrose by *C. pseudotropicalis*.

It is the feeling of the reviewer that the wealth of material is available, that the authors' long experience in the wards, clinics and laboratories of the Charity Hospital of New Orleans has fitted them for the task they set out to accomplish and that more careful attention to detail would have made this book more useful as a laboratory guide for the diagnosis of fungus infections.

NORMAN F. CONANT

***Biologie d'*Anopheles gambiae.** Recherches en Afrique-Occidentale Française. Serie de Monographies No. 9. by M. H. Holstein, Chargé de recherches de l'Office de la Recherche Scientifique Outre-Mer, Paris. (English edition in preparation). 176 pages. Geneva, World Health Organisation, Palais des Nations, 1952. 10/-; $2.00; Fr. fr. 640; Sw. fr. 8.

The author spent two years in French West Africa, mostly at Bobo-Dioulasso, which is in the Haute Volta savanna country. The article contains a summary of the published data on the biology of *Anopheles gambiae* and of the author's studies of certain aspects of the biology of that species. Some of the advice which is given is unduly elementary, but perhaps this must be viewed in the light of the fact that in his search of the literature the author could find only two published reports of dissections of *A. gambiae* for malaria parasites in French West Africa. Because the author's studies are largely ecological the lack of mention of tests for statistical significance is noteworthy. Not a single standard error is presented.

Among other things, the author investigated the potentially very important problem: are there two races of *gambiae*—zoophilic and anthropophilic? The criteria established appear to be of dubious validity and usefulness. Furthermore, the statement in the English summary that the zoophilic or "multidentate population . . . gave only very low anthropophilic indices" is not in agreement with the data in the body of the article. On page 133 it is stated that at least 34.8 per cent of multidentate *gambiae* captured in human habitations gave positive precipitin tests for human blood. In view of the efficiency of *gambiae*

as a vector of malaria this is quite obviously not a "very low" anthropophilic index. On page 145, Barber's figures for sporozoite indices are erroneously quoted in two instances. On page 139, Kerr is misquoted. The imposing list of 388 references loses much of its value because no titles are given. It is regrettable, to say the least, that WHO has sponsored a publication which is as untrustworthy as this one.

J. Austin Kerr

Expert Committee on Hepatitis. First Report. World Health Organization Technical Report Series No. 62 (Available also in French). 26 pp. Geneva, W.H.O., Palais des Nations. March, 1953. Price 1/6, $0.20, Fr. fr. 65.-; Sw. fr. 0.80.

This report presents in a compact and easily available form the current facts and theories concerning the two entities infectious hepatitis and serum hepatitis. The subject is covered in an orderly fashion beginning with a clarification of the terminology and discussion of the differentiation of the two diseases and the known characteristics of the viruses concerned (including their resistance to physical and chemical agents). Sections follow on methods of spread, epidemiology and control measures. The last three sections put forth the problems and possible solutions in the use of human blood and blood products and indicates areas in which further research on hepatitis is especially desirable.

The expert committee had notable success in its attempt to state briefly the existing knowledge regarding hepatitis. In doing so it includes two areas of comment not usually found elsewhere—a section on "Viruses Causing Hepatitis in Animals" and the suggested follow-up card to detect cases of serum hepatitis. The recommendation for compulsory notification (reporting) should meet with general approval.

Wm. Allen Longshore, Jr.

Malaria Terminology. Report of a Drafting Committee Appointed by the World Health Organization, by Sir Gordon Covell (Adviser on Malaria, Ministry of Health, Director, Malaria Laboratory, Horton Hospital, Epsom, Surrey, England), Paul F. Russell (Division of Medicine and Public Health, Rockefeller Foundation, New York) and N. H. Swellengrebel (Professor of Parasitology, University of Amsterdam, Director, Institute of Tropical Hygiene, Amsterdam, Netherlands). W. H. O. Monograph Series, No. 13. 82 pp., Geneva, Switzerland, World Health Organization. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York, 1953. Price 5/-, $1.00, Fr. fr. 320-, Sw. fr. 4.-

This is a needed revision of the *Report on Terminology in Malaria* published in 1940 by the Malaria Commission of the League of Nations and now out of print as well as out of date. New contributions to the knowledge of malaria have been many in the last twelve years and these have led to a more complete understanding of the parasite in the vertebrate and in the insect host, and of malaria infection in the human and in the anopheles community.

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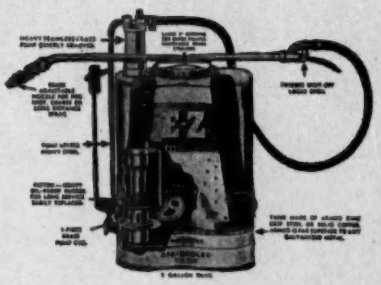

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